ADAPTING TO BEACH AND DUNE ENVIRONMENTS: A STUDY OF FITNESS AND ECOLOGICAL FACTORS IN THE COASTAL PLANT *CAKILE EDENTULA*

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Brittany Taylor Cole

Charlottetown, Prince Edward Island, Canada

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ABSTRACT

Species' geographical and ecological ranges are limited by the interaction of genotypes with the biotic and abiotic environment, and the quality of the habitat relative to the species' niche requirements. A fundamental question in ecology and evolution is how species will respond to changes in their environment; whether species will shift their distribution to track the niche environment, locally adapt, or become extirpated. Along an ecological gradient, the factors defining a species' limits and abundance are expected to vary. Species are defined by the interactions of genotypes with the environment, and these interactions may be constant in space with a strong genetic basis or vary plastically with the environment. This study assessed the phenotypic differences of *Cakile edentula* (sea rocket; Brassicaceae) plants along the beach to dune gradient within the species' Atlantic Canadian range, and attempted to disentangle whether plants growing along this gradient plastically respond or are locally adapted to their home microenvironment (beach vs. dune) within the coastal habitat. Empirical plant surveys in wild populations, and a greenhouse and a reciprocal transplant experiment all revealed significant phenotypic differentiation between beach and dune plants. The patterns of variation for most traits observed in the wild were not reflected in the controlled environment, suggesting plasticity rather than genetically based trait variation accounts for most variation observed in the wild. However, there was evidence that some traits (stem elongation and seed set) had a genetic basis as they varied consistently between beach and dune plants in all environments. A greenhouse experiment manipulating growing density and salinity exposure provided evidence that increased growth and fitness associated with wild beach plants compared to dune plants may be attributed, at least in

part, to the combined effects of plant density and sea spray. Moreover, this work supports the expectation for temporally and spatially variable environments that phenotypic variation in plants is primarily plastic, with some influence of maternal plant environment to offspring growth and fitness in the next generation. Overall, this study suggests *C. edentula* plants are highly plastic, and fitness variation between beach and dune plants likely indicates variation in habitat quality relative to the species' niche.

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My thesis is composed of two chapters. Chapter 1 is a literature review, and Chapter 2 is a data chapter outlining the projects of my thesis and their results.

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CHAPTER 1 – A LITERATURE REVIEW OF ECOLOGICAL LIMITS, THE MECHANISMS OF ADAPTATION, AND COASTAL HABITAT

1.1. INTRODUCTION

Biotic and abiotic conditions influence species' distributions and patterns of abundance on geographical (Cumming, 2002; Stanton-Geddes *et al.,* 2012) and ecological scales (Valladares *et al.,* 2007; Weber *et al.,* 2012). As conditions change across a habitat, so may a species' abundance, fitness and degree of adaptation to the habitat. One hypothesis explaining abundance patterns, the abundant centre hypothesis, suggests that species' thrive at the centre of their distribution and fade towards the edges (Brown, 1984); however, alternative abundance patterns have also been observed (e.g. Sagarin and Gaines, 2002; Samis and Eckert, 2007).

Adaptation to spatially and temporally variable environments plays a key role in the evolution of species across their geographic range, as well as within local habitats (Dudley, 1996; Guo *et al.,* 2005). The costs and benefits of plastic (passive phenotypic variation across environments for individual genotypes) versus genetic (phenotypes are predictable for each genotype regardless of environment) mechanisms of adaptation (two of the possible mechanisms) have been well studied under a variety of scenarios, but are less well understood at the limits of a species' range, and the relative contribution of each is generally not understood for most species (Sexton *et al.*, 2009; Eckert *et al.*, 2008;

Hargreaves *et al.*, 2014). Traditional models suggest phenotypic plasticity shields genotypes from selection (Levin, 1988), and may broaden the ecological range of individual genotypes (Callaway *et al.,* 2003) while limiting the potential of these genotypes to achieve high fitness in any particular environment (van Kleunen and Fischer, 2005). However, alternative models indicate that plasticity may also be adaptive, such that optimally suited plastic phenotypes are selected for in new environments and when phenotypes confer higher fitness relative to other local species (Bradshaw, 1965; van Kleunen and Fischer 2005; Baythavong, 2011). It is well accepted that phenotypic plasticity and local adaptation likely contribute to the evolution of species' ecological and geographic ranges (Valladares *et al.,* 2007; Ghalambor *et al.,* 2007), but the extent to which each contributes and under what ecological conditions is less clear.

Phenotypic plasticity, or variation in phenotype for a given genotype across growing environments, is common in plants (Sultan, 2003; Nicotra *et al.,* 2010; Valladares *et al.,* 2007). Plasticity is important in sessile organisms, including plants, as a means to persist in variable habitats and since individuals cannot avoid changes in their environment (Schlichting, 1986). Although dynamic habitats favour organisms displaying phenotypic plasticity (Williams *et al.,* 1995), many species evolve specialized traits adapted for growing in their niche (Richards *et al.,* 2010); i.e. specialist, non-plastic genotypes. These adapted traits typically confer high fitness and heritability, meaning their expression varies little between generations or among microhabitats. Furthering our understanding of phenotypic plasticity vs. local adaptation in spatially and temporally variable habitats, particularly in study systems where both processes likely play a role, is important to ecological research. Learning how different evolutionary mechanisms enable

individual species to survive in their habitats will allow better management of species' distributions and their habitats with climate change.

In this thesis, I investigated the effects of the coastal environment and zonation on plant fitness and phenotype, using a common coastal plant, *Cakile edentula* (Brassicaceae). This literature review (Chapter 1) will first discuss ecological limits and mechanisms of adaptation, particularly as they pertain to coastal habitats, followed by a description of the study habitat and study system. Finally, I present my thesis objectives and an overview of the thesis organization.

1.2. ECOLOGICAL LIMITS

Species' geographical and ecological ranges are limited by the interaction of genotypes with the biotic and abiotic environment, and the quality of the habitat relative to the species' niche requirements (Stanton-Geddes *et al.,* 2012; Gómez *et al.,* 2004). The factors determining geographical ranges (Sexton *et al.,* 2009; Eckert *et al.,* 2008; Hargreaves *et al.,* 2014; Hargreaves and Eckert, 2014, and references therein), and how geographic range limit theory applies to ecological limits, remain poorly understood for most species (Geber, 2008). One approach to understanding geographical limits may be to assess ecological limits (Angert, 2009). It is expected that the most important determinants of a species' geographic range are those changes and interactions that occur at a fine spatial scale immediately at the range edge; i.e. factors occurring at the ecological level (Antonovics, 1976; Samis and Eckert, 2009; Bridle *et al.,* 2010).

There is a center and edge to every spatial distribution (Brown, 1984). The edge of a species' range may be a hard boundary, such as the edge of land into water, but most distributional edges occur along continuous environmental gradients, i.e. transitional edges (Gaston, 2003). Despite complexities, high latitude and elevation limits are often, but not always, associated with changes in climate (e.g. tree lines), while lower limits are often associated with biotic interactions (Hargreaves *et al.*, 2014). For example, the high altitudinal distribution of *Oncorhynchus clarkii utah* (Salmonidae; cutthroat trout) has been attributed to temperature and the lower limit to competition with exotic *Salmo trutta* (Salmonidae; brown trout) (McHugh and Budy, 2005). In contrast, *Potentilla diversifolia* plants (Rosaceae; different-leaved cinquefoil) showed little to no effect of biotic interactions (in this case, on population density) at either end of their altitudinal distribution, instead abiotic factors (temperature and soil moisture) reduced survivorship and reproduction when transplanted below the native range (Stinson, 2009).

From an ecological perspective, a niche is defined as the ecological conditions that match a species' requirements for growth, survival, and reproduction, such that populations are self-sustaining. A fundamental niche describes habitat in which birth rates exceed death rates, and may occur outside of a species' observed geographic range. In contrast, the realized niche refers to habitat occupied by a species and matches their geographic distribution (Hutchinson, 1957; Holt and Gaines, 1992). In this thesis, the terms distribution or geographic range reflect a species' realized niche, while the term niche refers to the observed geographic range plus available, yet unoccupied habitat (i.e. "niche" = the fundamental niche).

At large geographic scales, a species' realized niche is often smaller than the global distribution of their fundamental niche due to dispersal constraints and barriers (Pulliam, 2000). For that reason and because of the difficulty quantifying fine scale variation, defining a species' niche is a difficult task (e.g. Angert, 2009; Kanda *et al.*, 2009; Marsico and Hellmann, 2009). However, transplant experiments and controlled greenhouse experiments designed to measure local adaptation and phenotypic plasticity are one step to determining the spatial and ecological extent of a species' habitat. Hargreaves *et al.* (2014) reported in a meta-analysis of transplant experiments conducted beyond species' geographical range limits that while most species' ranges appear to coincide with their niche limit (i.e. fitness declines beyond the range edge when experimentally moved there; niche-driven range limits), a substantial number of species' ranges occur within the boundaries of their niche, such that experimental plant fitness does not decline beyond the range edge (26% of 111 experimental tests of range limits reviewed in Hargreaves *et al*., 2014).

Niche limits that exceed species' distributional limits are best explained by dispersal constraints (Figure 1 in Hargreaves *et al.*, 2014). For example, a transplant experiment demonstrated that the absence of montane populations of *Euphydryas gillettii* butterflies (Lepidoptera) in similar habitat beyond the current distribution limits in Wyoming, USA was not due to the lack of suitable niche conditions but rather limitations to long-distance dispersal preventing migration across a large water basin (Holdren and Ehrlich, 1981). Similarly, changes in abundance of woody and herbaceous forest plant species occurring within a continuous gradient from natural to artificial forest along a Japanese coastline demonstrate how species adapted for passive dispersal (e.g. via wind

or gravity) are more dispersal limited than species using active forms of dispersal (including ingestion by herbivores or adhesives) (Takahashi and Kamitani, 2004). Takahashi and Kamitani (2004) reported that the forest species using active modes of dispersal were able to migrate and establish new colonies in the artificial forest while those using passive dispersal were not. Hence, dispersal mechanisms are influential not only in extending (or limiting) species' ranges, but also determining community structure in newly available habitat.

When distributional limits coincide with niche limits, fitness and abundance are predicted to decline from the centre to the range edge (Brown, 1984; Hoffmann and Blows, 1994). Experimental tests of fitness across range limits support this assertion in 46% of 111 tests (revealed fitness declines beyond the range edge) reviewed by Hargreaves *et al*. (2014). However, the corresponding decline in abundance from the center to edge of the range, as predicted by the abundant center hypothesis (assumes a positive correlation between abundance and fitness; Brown, 1984), is not often reported. In addition, there are often discrepancies in fitness and abundance patterns between theoretical (and traditionally held) expectations and empirical data (Sagarin and Gaines, 2002). A favourable site where population density and plant fitness should be greatest because the habitat meets niche requirements may occur anywhere within a species' range (Brown, 1984), and this pattern has been observed for numerous species. For example, the coastal plant *Camissonia cheiranthifolia* (Onagraceae, beach evening primrose) was reported to have highest plant density in populations occurring outside the geographic range centre (Samis and Eckert, 2007). Other examples of highest abundance at range edges were reported for several species of intertidal invertebrates native to the

coastline of California (Sagarin and Gaines, 2002; Gilman, 2006). While these examples appear to occur in species with stable range limits, when range edges vary temporally it becomes more difficult to associate current patterns of fitness and abundance with range limiting factors (Channell and Lomolino, 2000). As habitats or climates change, natural selection on dispersal at range edges may provide the opportunity for range expansion. For example, several species of non-migratory European butterflies (63% of 35 species) have shifted their ranges north over the past century in response to rising temperatures (Parmesan *et al.,* 1999).

Some of the most interesting cases of examining range limits are those where the species' range extends beyond their apparent niche limits into sink habitats. Sink habitats may provide the resources and conditions required for growth and survival, but not for reproduction, such that populations occurring in these habitats are not self-sustaining. For example, after observing the highest *Cakile edentula* fitness and total seed production on the beach but the highest abundance in the dunes (i.e. an inverse correlation), Keddy (1980) hypothesized that dune sub-populations likely persisted at higher levels of abundance than local seed production supported due to seed supplementation via dispersal from beach sub-populations (asymmetric dispersal). Although this observation in *C. edentula* is a well-cited example of demographic sinks, it appears that most experimentally tested cases of range edge populations persisting as demographic sinks occur at elevational range limits (Hargreaves *et al.,* 2014).

The most extreme example of a source-sink dynamic is termed a black-hole sink, and describes a situation where a poor quality, sink habitat is strictly supported by dispersal from a good quality habitat, and where death rates are substantially greater than

birth rates (Holt and Barfield, 2011). The black-hole sink scenario could be a mechanism through which species' expand their distributions outside the historical niche range and may allow for niche evolution via natural selection when new traits arise in habitats that the species was not previously adapted to (Holt and Barfield, 2011; in contrast to niche conservatism, which may limit range expansion and was reviewed in Wiens and Graham, 2005). Repeated introduction of non-native species that eventually establish in their introduced range (Dlugosch and Hays, 2008) may be an example of range expansion through niche evolution in habitat that was initially a demographic sink. Niche evolution could also arise through the evolution of mutations required to live in non-traditional habitats, or the occurrence of phenotypic plasticity.

In summary, all species have an ecological niche, but the relationship between the niche and the distributional limits may vary with spatial and temporal variation in the environment. The mechanism employed to overcome environmental variation can be uncovered through our understanding of genetic adaptation versus plasticity.

1.3. MECHANISMS OF ADAPTATION

A fundamental question in ecology and evolution today is whether species will shift their geographic ranges to track their niche environment or adapt locally in response to changing environments. Unless ecological factors remain within the realm of conditions to which the species is already adapted, it seems likely that species unable to respond to climate change will go extinct in the absence of evolution or range shifts. For most species, answering this question requires an understanding of the two strategies that

account for intraspecific levels of phenotypic variation within and among habitats: genetic adaptation and phenotypic plasticity (reviewed by Gienapp *et al.,* 2008).

Organisms are defined by the interactions of genotypes with the environment, leading to phenotypic variation across the landscape. Selection on dispersal and adaptation to the changing environment are particularly important in sessile organisms (plants and non-migratory animals) (Venable and Brown, 1988). Adaptation may result from natural selection on phenotypes that confer higher fitness than other phenotypes in a given environment. More specifically, local adaptation refers to population level adaptation that occurs when genotypes have higher fitness at-home than away-from-home and higher fitness at-home than foreign genotypes. The occurrence of local adaptation can be assessed using reciprocal transplant experiments (Kawecki and Ebert, 2004). Local adaptation has been demonstrated repeatedly in the literature (reviewed by Hereford, 2009), and is particularly common between strongly differentiated habitats, such as between serpentine and non-serpentine soils (e.g. *Helianthus exilis*, Asteraceae, serpentine sunflower; Sambatti and Rice, 2006) or coastal and non-coastal habitats (e.g. *Mimulus guttatus*, Phyrmaceae, yellow monkey flower; Hall and Willis, 2006).

Genotypic variation may also be structured such that individual genotypes confer high fitness in more than one habitat (at-home and away-from-home), which would not support a hypothesis of local adaptation. For example, a reciprocal transplant experiment with *Suaeda maritima* (Amaranthacea, annual seablite) plants between aerated and hypoxic areas of a salt marsh revealed little evidence of local adaptation (Wetson *et al.,* 2012). While the morphology of plants growing in the aerated soil was larger and more branched than plants growing in the hypoxic soils, sibling fitness in non-native areas was

similar to the high fitness recorded at-home in both genotypes (Wetson *et al.,* 2012). This interaction between genotype and environment is called plasticity, which is defined as the expression of more than one phenotype by a single genotype (reaction norms) (Gomulkiewicz and Kirkpatrick, 1992).

When environmental variation occurs over a large scale (e.g. foothills to mountains to valleys) relative to individual dispersal distances, individuals are likely to experience a limited range of biotic and abiotic conditions. Under this scenario, selection remains consistent across generations, offspring experience the same environment as their parents, and local adaptation is likely to occur (Storz *et al.,* 2010). However, when environmental variation occurs over a fine scale (e.g. soil moisture and canopy density across the forest floor) relative to individual dispersal distances, individuals are likely to experience a broad range of conditions and offspring may not occur in the same environment or habitat patch as their parents (Storz *et al.,* 2010). Whatever the spatial scale at which ecological variation occurs, offspring are more likely occurring in habitats that vary from the maternal environment, and may require the plastic expression of traits to thrive in this new environment when dispersal distances are greater than the scale at which habitat variation occurs.

Plasticity can have neutral or adaptive effects on a species' evolution, or maladaptive effects on the rate of evolution (Callaway *et al.,* 2003). Passive phenotypic plasticity occurs when variation in phenotype is not due to variation in gene regulation but rather a non-genetic effect of the environment on a phenotype. For example, when nutrient levels are low, individuals may grow smaller than when nutrient levels are high – that is, the reduction in growth is a passive response to the habitat. While the expression

of phenotypic plasticity is often passive, plasticity may also be adaptive when natural selection acts on an optimal phenotype in a given environment (Valladares *et al.*, 2007). In theory, adaptive plasticity confers high fitness in individuals expressing phenotypes that are favourable in a given environment, and that fitness associated with this phenotype is greater than fitness associated with other phenotypes conferred by the same genotype (van Kleunen and Fischer, 2005). In the wild, it is exceptionally difficult to distinguish adaptive plasticity from passive given that plasticity leads to the most optimal phenotype; hence comparisons to non-optimal phenotypes are unlikely to occur (Schmitt *et al.,* 1999; van Kleunen and Fischer, 2005).

Introduced, Hawaiian *Pennisetum setaceum* (Poaceae, fountain grass) may provide an example of adaptive phenotypic plasticity (Ghalambor *et al*., 2007). In field observations, plants from three distinct environment types expressed distinct phenotypes creating phenotypically differentiated populations (Williams *et al.,* 1995). However, reciprocal transplant experiments revealed little evidence of genetic differentiation for these traits (e.g. plant height, net photosynthesis, and seed production), supporting the adaptive plasticity hypothesis. While *P. setaceum* may provide an example of adaptive plasticity, in this case the lack of genetic differentiation would suggest no opportunity for selection. That is, plasticity is adaptive with production of highly optimal phenotypes in each environment, but prevents genetic differentiation and trait evolution (Ghalambor *et al.,* 2007).

Plasticity is maladaptive when a phenotype results in reduced fitness. Human acclimatization response to hypoxia provides an example of maladaptive plasticity – our body responds to chronic low oxygen levels by increasing hematocrit concentration and

decreasing hemoglobin-oxygen affinity, which are counterproductive hematological adjustments (in the opposite direction of the phenotypic optimum) under severe hypoxia that may result in death (Storz *et al.,* 2010).

An organism's phenotype is not only determined by the genotype and environment, but also by environmentally based maternal effects that occur beyond genetic contributions. The maternal environment may influence offspring directly, such as through resource allocation to developing seed endosperm (early life nutrient source occurring before root development) in plants. Plants growing in nutrient rich environments are expected to provision more resources to seeds than plants growing in nutrient poor environments (e.g. Zhang, 1996; Donohue, 2009). As with phenotypic plasticity, maternal effects may be passive, adaptive or maladaptive. The type of maternal effect typically depends on the correlation between maternal and offspring habitat and fitness, and genetic variation in maternal effects (Donohue and Schmitt, 1998). In the case of wild semelparous sockeye salmon (*Oncorhynchus nerka*), mothers exposed to chase stressors produced offspring with phenotypes geared to burst swimming, which is advantageous for escaping predators and may indicate adaptive maternal effects (Sopinka *et al.,* 2014). As an example in plants, Donohue (1998b) reported that in *Cakile edentula,* plastically reduced maternal plant growth resulted in reduced maternal plant fitness (low seed production), but also increased offspring (seed) dispersal distances compared to distances measured from large maternal plants. This apparent trade-off was hypothesized to be a method for dispersing offspring to better habitat than the maternal plant experienced.

Plants are an ideal study system for researching adaptation because of their sessile nature. Numerous plant traits have been reported as plastic, including traits related to morphology, physiology and anatomy, development and reproductive timing, breeding systems, and offspring development (Sultan, 2000). One of the well-studied examples of phenotypic plasticity in plants is stem elongation in response to light cues (Valladares *et al.*, 2007), which has been reported as adaptive (e.g. Dorn *et al.,* 2000) and maladaptive (e.g. Weinig, 2000).

Ecologists and evolutionary biologists have long been intrigued by how species inhabit various environmental conditions and how species regulate phenotypic variation across environmental gradients. Coastal habitat provides an excellent example of a spatially and temporally variable environment, and offers a narrow, but dynamic system within which we can assess phenotypic variation in plant populations.

1.4. COASTAL HABITAT

Coastal habitat occurs on the edge between terrestrial and marine systems, with abrupt water's edge contrasted by a gradual inland transition to progressively stabilized vegetation. This narrow terrestrial habitat provides an opportune setting to assess fitness, distribution, abundance, and the mechanisms of adaptation for coastal plants, especially coastal endemics. Coastal habitats and zonation patterns from beach to dune have been well documented around the world (e.g. Oosting and Billings, 1942; Wilson and Sykes, 1999; Maun, 2009) and an introduction to this ecological environment is provided here.

Sandy coasts make up two-thirds of the world's ice-free coastlines, and are harsh habitats often experiencing the brunt of extreme weather with high winds, wave inundation, substrate movement, and salinity (McLachlan and Brown, 2006; Maun, 2009). For these reasons and because of physiological and evolutionary factors that may constrain species from inhabiting coastal habitat, relatively few species are endemic to coastal habitat compared to most other inland terrestrial habitats (e.g. Oregon and California coastal dunes: Samis and Eckert, 2009; Isle de la Madeleine, Quebec: Houle, 2008).

A common feature of sandy coastlines is the environmental gradient stretching from the intertidal zone to vegetated dunes (Keddy, 1982; McLachlan and Brown, 2006). For the purpose of this thesis, the coastal habitat is separated into two relatively distinct zones (excluding the intertidal zone where typically there is no terrestrial vegetation): mid-beach and dune. These two zones are known to vary in environmental conditions that influence the distribution, abundance, and survival of coastal vegetation (Oosting and Billings, 1942; Forey *et al.,* 2009).

The mid-beach (herein referred to as beach) is the area in-between the intertidal zone and the dune. Beach habitat favours annual, salt-tolerant plants because of their short life cycle, high growth rates, and reproductive prolificacy, which are suitable traits in a highly fluctuating habitat. Common mid-beach plants in Atlantic Canada include *Honckenya peploides* (Caryophyllaceae, sandwort) and *Cakile edentula* (Brassicaceae, sea rocket). In contrast to the beach, the dune zone on sandy coastal sites backed by sand dunes (other local coastal sites are backed by till bluffs or sandstone cliffs; MacMillan and Quijón, 2012) is formed by wind-blown sand that primarily collects around

vegetation (Maun, 2009). Dunes typically extend inland from the beach to areas such as a forested ridge, agricultural land, or salt marsh (Maun, 2009). Dune habitat can usually be separated into three successional microhabitats: foredune (primary dune), established dune, and backdune. The foredune, the first dune inland from the beach, may contain grasses, forbs, and herbs (e.g. *Lathyrus japonicas* [Leguminaceae, beach pea], *Lechea intermedia* [Cistaceae, beach pinweed], and *Limonium carolinianum* [Plumbaginaceae, sea lavender]), and is often reshaped by winter storms and wave action (Maun, 2009). Grasses have been associated with reducing airflow (Hesp, 2002), trapping sand and changing the structure of the dune (Seneca *et al.,* 1976), as well as influencing seed dispersal, fitness, and survival of other plants (Pemadasa and Lovell, 1974a,b). For the purposes of this research, the foredune microhabitat also includes embryo dunes, which consist of unconnected mounds of dune-like habitat on the beach that are mainly vegetated by perennial dune grasses with stolons or plagiotropic (horizontal-growing) rhizomes, such as *Ammophila breviligulata* (Poaceae, marram) and *Leymus mollis* (Poaceae, wild rye) (Maun, 2009). The established dune is more stable than the foredune with more dense cover of vegetation and greater diversity of perennial species, such as woody shrubs (e.g. *Myrica pensylvanica* [Myricaceae, northern bayberry] and *Hudsonia tomentosa* [Cistaceae, beach heather]). The established dune can consist of several subsequent ridges, beginning as early successional foredunes and transitioning into established dunes. Finally, the backdune typically includes the transitional area between coastal dune and adjacent terrestrial habitat. While *C. edentula* may occur in the backdune and established dune, abundance there is generally low and thus represents

individuals existing at the edge of the species' ecological niche. This research focused on the foredune, which herein is referred to as the dune, and the beach as described above.

Two commonly assessed abiotic determinants of coastal zonation (a sequence of areas occupied by distinctly different plant communities; Maun, 2009) are sand burial and salinity (Oosting and Billings, 1942; Wilson and Sykes, 1999; Maun, 2009). Sand accretion occurring in coastal habitat is the result of seabed sand transported inland by near shore wave action and wind deposits (McLachlan and Brown, 2006). While typically plant burial by sand reduces with distance from the water's edge (Davidson-Arnott and Law, 1990), sand deposition remains unpredictable. This dynamic and evolving habitat is challenging for species with specialized phenotypes that cannot tolerate burial (plastically respond or locally adapted to burial) (Maun, 2004). A study employing artificial burial (Maun and Perumal, 1999) reported variation in burial tolerance among coastal species with the majority of annual plants in the study. *Corispermum hyssopifolium* [Amaranthaceae, tumble weed]*, C. edentula,* and *Strophostyles helvola* [Fabacea, wild bean]) did not emerge above the substrate after buried at depths > 15 cm. In contrast, some perennials in the study (*Poa compressa* [Poaceae, Canada bluegrass] and *Agropyron psammophilum* [Poaceae, wheat grass]) were eliminated after burial to depths between 40-80 cm, while another perennial grass, *Ammophila breviligulata*, was still abundant after 80 cm of burial (Maun and Perumal, 1999). In another example, coastal moss bryophytes colonizing the foredune (*Ceratodon purpureus* and *Ditrichum flexicaule* [Ditrichaceae]) were more tolerant of burial (greater emergence frequency and speed, and a lower reduction in percent cover above ground) than moss bryophytes colonizing inland habitat at the base of trees (*Dicranum scoparium* and *D. flagellare* [Dicranacea],

Brachythecium sp. 2 [Brachytheciaceae], *Plagiomnium cupidatum* [Mniaceae]) (Martínez and Maun, 1999). Taken together, these data suggest coastal plants may differ in the amount of burial tolerated, but overall it is clear that coastal species are adapted to sand burial.

Salinity variation in the coastal habitat is mainly dependent on wave action and sea spray. Seawater (a weak nutrient source for plants) typically contains Ca^{2+}, K^{+}, Mg^{2+}, Cl^{-}, SO_4^{2-}, and Na^{+} as the most abundant essential nutrients available to plants, and CO_2, NO_3^{+}, and NH_4^{+} as moderately abundant (Brown *et al.,* 1989), however not all plant species tolerate seawater exposure because of a sensitivity to high salt concentrations. Coastal plants are highly tolerant to sea spray and have developed several mechanisms for dealing with high salinity (Boyd and Barbour, 1986; Debez *et al.,* 2004). Sea spray deposition declines with increasing distance from the tide line (Oosting and Billings, 1942; Barbour, 1978; Maun, 2009), and appears to influence vegetation zonation. For example, a study on herbaceous plants native to New Zealand sand dunes suggested sea spray tolerance influenced the distributions of *Desmoschoenus spiralis* (Cyperaceae, golden sand sedge) and *Bromus diandrus* (Poaceae, great brome) (Sykes and Wilson, 1988). However, in another study it was reported that genotypes of a salt marsh plant, *Borrichia frutescens* (Asteraceae, sea oxeye), were not locally adapted to high versus low salt levels along the salinity gradient that this species generally inhabits (4 – 127 ppt) despite the diverse range of phenotypes along the gradient (Richards *et al.,* 2010).

Variation in substrate salinity is attributed to wave inundation and sea spray, however sandy substrates are not considered saline because sand does not easily retain salt and rainfall typically leaches salt out of the rhizosphere (Maun, 2009). For example,

most global sandy coastal substrate salinity levels range from 0.003-0.13% (e.g. Fremantle, Australia 0.005-0.009% saline, Olsson-Seffer, 1909; Barbados, West Indies 0.0064-0.013% saline, Gooding, 1947; Norfolk, Virginia 0.003-0.009% saline, Kearney, 1904). In contrast to low substrate salinity levels in the natural habitat a common coastal plant, *Cakile edentula*, has been shown in experimental studies to tolerate up to 2.9% salinity, well above the maximum observed in coastal habitats (Olsson-Seffer, 1909).

In summary, variation along an environmental gradient is often due to interactions between several biotic and abiotic factors (Wilson and Sykes, 1999; Levinsh, 2006), making the beach-to-dune gradient a suitable habitat for assessing plant adaptation strategies. Testing genetic vs. plastic modes of trait variation between beach and dune plants may confirm whether plants in the coastal habitat are highly plastic, the expected mode of adaptation in variable habitats, or whether they show traits that are more genetically based. However, gaps in our understanding of plant ecology along coastal gradients mean we can only speculate which beach and dune characteristics structure coastal endemic populations. Additional studies on the influence of coastal zonation on species abundance and distribution are necessary for predicting plant response to the evolving coastal habitat.

1.5. STUDY SYSTEM

Plants are an excellent study system for assessing adaptation to environmental variation because they are sessile, the mechanisms for adaptation in plants have been well documented (see examples above), and they are often easy to grow, manipulate, and

gather life history and fitness data on. Coastal plants are especially advantageous to research on ecological adaptation, because they survive along the distinct and narrow environmental gradient from beach to dune – a well described and informative habitat type for assessing adaptation.

The study system for this project is a common coastal plant, *Cakile edentula* ssp. *edentula* var. *edentula* (Brassicaceae, sea rocket), which has an Atlantic coastal range extending from North Carolina, USA to Labrador, Canada (Rodman, 1974). *Cakile edentula* is an annual plant that typically germinates in the spring and senesces after reproduction in late-fall (Rodman, 1974). The plant is characterized by having typical, small flowers for plants in the Brassicaceae (mustard) family, and with succulent leaves growing alternately on stems. Flowers are self-compatible and reproduction is likely through self-pollination. Flowers produce dimorphic fruit with two halves; proximal and distal. The larger, bulbous distal segments detach for dispersal away from the maternal plant, while the smaller proximal segments usually remain attached and create a seed shadow around the maternal plant (Keddy, 1982, Donohue 1997). Having dimorphic fruit with different dispersal strategies for each morph is advantageous in spatially and temporally variable environments as it increases the probability of offspring success. Although each fruit segment typically produces one seed, the distal segment produces a seed more often than the proximal segment (Keddy, 1982, K.E. Samis *unpublished data*).

Cakile edentula habitat stretches from open sandy beach to densely vegetated dunes (Rodman, 1974) with the greatest plant abundance occurring on the first dune ridge behind the beach, the foredune (A2.1 Figure 1; Keddy, 1982), and abundance declining in both directions from this ridge onto the beach and further inland. However, plant

abundance within the environmental gradient may vary between populations with variation in biotic and abiotic conditions (Keddy, 1982). *Cakile edentula* is one of the few terrestrial plants to establish close to or at the tidal line (Barbour, 1970).

Local adaptation in plants is best assessed by quantifying survival and fitness in native and non-native habitat using reciprocal transplant experiments (Stanton-Geddes *et al.,* 2012; Holt and Gaines, 1992; Samis and Eckert, 2009), but few experiments have measured local adaptation in *C. edentula* along the beach to dune gradient. Fitness of *C. edentula* can be measured by quantifying fruit production and seed set between the two fruit halves (expectation of one seed per fruit half). Since *C. edentula* is primarily self-pollinating, all individuals should have equal opportunity to reproduce, which is a beneficial reproductive method for experimentally measuring fitness as it reduces variation introduced by pollinators. Despite a predominantly selfing mating system, not all *C. edentula* flowers produce two viable seeds (Donohue, 1998a). A plant producing a high proximal to distal seed set ratio (when both seed halves from the same flower produce one seed each) appears more frequently in a favourable habitat, while a low ratio (more distal than proximal seeds) occurs in plants growing in poor quality habitat (K.E. Samis *unpublished data*; Keddy, 1982). Discrepancies between seed set in proximal and distal fruit may indicate a maternal plant strategy to improve offspring fitness through dispersal to potentially better quality habitat.

Theoretical models suggest dispersal is not a selectively neutral character and dispersal from the maternal plant influences offspring fitness (reviewed in Howe and Smallwood, 1982). Dispersal in Brassicaceae plants has been well studied. Experimental studies by Donohue (1998b; *C. edentula* ssp. *edentula* var. *lacustrus*) and Wender *et al.*

(2005; *Arabidopsis thaliana*) have demonstrated that maternal plant morphology and growing density affect the dispersal potential of offspring. For example, *Cakile edentula* offspring dispersal distances were impeded by tall maternal plants with few branches and fruit on the primary stem, leading to more dense seed shadows compared to short maternal plants with more primary branches (Donohue, 1998b). However, high offspring density is also associated with reduced plant growth through sibling competition (Bhatt *et al.,* 2011), suggesting that sibling competition is indirectly determined by selection on maternal plant morphology.

Plant density may also be correlated with environmental factors, which can make identifying density-dependent versus density-independent effects difficult in the wild. Natural populations of *Cakile edentula* have been observed to have highest density in the foredune, which is the middle of its spatial distribution in the coastal habitat; however, density has the greatest impact on fitness at the seaward end of the gradient (Keddy, 1982). Keddy (1982) suggested the abundance patterns of *C. edentula* are caused by asymmetric dispersal (source-sink) of fruit landward from the beach and supplementing the seed bank in the dunes. If *C. edentula* is locally adapted to the beach, then landward dispersal of alleles not adapted to the dune, rather than the effect of density, may be the main constraint of fruit production in dune described by Keddy (1982).

Variation along the coastal ecological gradient has been well documented and some of the main factors, including salinity, are discussed above. In a classical study by Oosting and Billings (1942), the significance of salinity on the local distribution of coastal species was revealed – as the levels of airborne salt spray decrease with increasing distance inland from the tide line, the presence of species susceptible to sea

spray increases. Studies have indicated that shoreline species, such as *C. edentula*, are largely unaffected by natural airborne salt levels (Barbour, 1970; Boyd and Barbour, 1986; Ellouzi *et al.,* 2011). *Cakile edentula* tolerates salt by enlarging cells, referred to as hypertrophy, and having high ion uptake to maintain turgor (Maun, 2009). Succulent plants like *C. edentula* plastically respond to salt by accumulating salt ions in their vacuoles (a strategy referred to as compartmentalization), restricting the accumulation of sodium and chloride ions in the cytoplasm, and shedding leaves when salt ion accumulation becomes toxic (Maun, 2009).

The biology of *Cakile edentula* and its relatives have been well documented, however the ecology of *C. edentula* in coastal habitat and its niche requirements are still under review. This species contributes to the structure of our coasts and thus is an important system for research. *Cakile edentula* was used for this thesis research to study the mechanisms of adaptation along the coastal ecological gradient at the edge of terrestrial habitat.

1.6. RESEARCH OBJECTIVES

The overall goal of this research was to characterize the association in the study system, *Cakile edentula*, between ecological and fitness variation across the narrow habitat range from the water's edge into the coastal dunes. The first objective was to quantify phenotypic and fitness variation between zones (beach vs. dune) within several wild populations in Atlantic Canada. The second objective was to assess whether plants were locally adapted to beach or dune microhabitats using a reciprocal transplant

experiment within and between two sites with seed collected from wild plants. Third, experimental manipulations of abiotic and biotic factors were employed in the transplant experiment to measure genotype by environment interactions within and among habitat zones. The final project objective was to assess the effect of two putatively important ecological factors, plant density and salt spray exposure, under environmentally controlled conditions with a greenhouse experiment using seed collected from wild populations. Although maternal environment factors (beach vs. dune source, and site of origin) may contribute to offspring performance under experimental conditions, these experiments provide some indication of the influence the maternal environment has, from which the potential success of offspring dispersing from beach vs. dune zones may be interpreted.

Ultimately, it was hypothesized that *C. edentula* would show high plasticity for most traits, but that fitness would be higher in plants growing on the beach or grown from seed collected from the beach zone than for plants from the dune zone due to increased local and maternal habitat quality relative to the species' niche requirements. *Cakile* species are adapted to the coastal habitat and thrive on the beach, and nearest to the marine edge of the species' ecological distribution. Individuals growing outside or moved inland from the beach, both ecologically and spatially, are expected to display reduced fitness through plastic variation in plant growth and exposure to a sub-optimal environment.

CHAPTER 2 – PLANT ADAPTATION TO ECOLOGICAL LIMITS AND THE COASTAL ENVIRONMENTAL GRADIENT

2.1. INTRODUCTION

Species' geographic range limits have been well studied (e.g. Kirkpatrick and Barton, 1997; Holt, 2003; Bridle and Vines, 2007; Gaston, 2009; Geber, 2011), but the application of range limit theories to local scales requires further attention. Species' geographical and ecological ranges are limited by the interaction of genotypes with the biotic and abiotic environment and the quality of the habitat relative to the species' niche requirements (Stanton-Geddes *et al.,* 2012; Gómez *et al.,* 2004). Therefore, the influence of abiotic versus biotic conditions may vary along an environmental gradient (MacArthur, 1972), and how these interactions structure species' range limits may vary among species and habitats.

A fundamental question in ecology and evolution is whether species will shift their distribution to track their niche environment, adapt locally in response to environmental change, or become extirpated. Adaptation is the potential outcome of natural selection on phenotypes associated with genotypes conferring higher fitness in specific habitats (typically the maternal environment). In contrast, phenotypic plasticity is the interaction between genotypes and the environment leading to several phenotypes for a single genotype, which allows for survival in variable habitats (often away from the

maternal environment) (Ghalambor *et al.,* 2007). In addition to the mechanisms of adaptation, maternal effects also influence offspring's response to environmental factors, and may even determine the environment offspring experience (e.g. Donohue, 1998b).

Selection on dispersal and adaptation to the changing environment are particularly important in sessile organisms, such as plants (Venable and Brown, 1988). Plants are highly plastic, which is a favourable trait since plants cannot choose their environment (Valladares *et al.,* 2007). However, given that some species' range limits do not coincide with their niche limits, ecologists continue to question what ecological and evolutionary factors determine species' distributions.

This study assessed the factors that contribute to the local ecological distribution of *Cakile edentula* plant populations within the Atlantic Canadian (Maritime) coastal range, and provides data on whether plasticity or local adaptation defines the phenotypic differences between beach and dune plants. A combination of empirical surveys on wild populations and controlled experiments (transplant and greenhouse) was used to assess the effects of important demographic and ecological factors (location, density, burial, and salinity) on phenotypic and fitness variation observed in the wild.

There are obvious, yet subtle habitat differences between beach and dune habitats, including the influence of sea spray, and higher plant abundance in the dune compared to the beach. Based on previous research (e.g. Keddy, 1982; Payne and Maun, 1984; Zhang, 1996), it was hypothesized that *Cakile edentula* plants growing on the beach would have higher fitness than dune plants regardless of maternal environment since the dune habitat is believed to be less favourable than the beach. Additionally, it was hypothesized that morphological traits (e.g. size and number of branches) associated with fitness would

differ between beach and dune, because, for example, a large heavily branched beach plant may not perform as well in the dune as on the beach (e.g. Keddy, 1982; Donohue, 1998b).

Classical literature and more current studies report that *Cakile* is highly resistant to salt spray (Boyce, 1951; Martin, 1959; Boyd and Barbour, 1986; Debez *et al.,* 2004), dealing with salinity through the abnormal enlargement of cells to maintain turgor (hypertrophy) and shedding of old leaves. Based on Keddy's (1982) conclusion that beach plants had higher fitness than dune plants, our experimental *C. edentula* plants exposed to seawater spray (a weak nutrient source more frequent in the beach than dune habitat; Oosting and Billings, 1942), were expected to have higher fitness regardless of seed source habitat and density. It was also expected that salt-sprayed plants would plastically respond to the influx of salt by increasing leaf turnover and succulence.

While many studies have highlighted density-independent effects along the beach to dune ecological gradient (e.g. as reviewed in Maun, 2009), density-dependent fitness and survival are also ecologically relevant and may involve interactions with density-independent factors (e.g. Pemadasa and Lovell, 1974a; Xu and Yu, 2014). Increased congener plant density, often observed in *C. edentula* foredune distribution, was hypothesized to reduce fitness and size of beach and dune sourced experimental plants (i.e. plants grown in the greenhouse from seeds collected from wild plants) due to resource competition. While Keddy (1981) reported density-dependence of fitness to be greater in plants at the seaward end of the gradient than dune plants, it was expected that our dune sourced experimental plants would be less stressed (have higher fitness) in high density than beach plants. Studies on plant stem elongation response to increased growing

density (Dudley and Schmitt, 1996; Alokam *et al.,* 2002) lead to the hypothesis that stem length would also increase for all of our experimental plants growing in high density as a possible response to reduced light caused by close neighbours.

Finally, conspecific maternal plant density and the surrounding environment may influence offspring density through maternal plant fruit dispersal (Thiede and Augspurger, 1996; Wender *et al.,* 2005). *Cakile edentula* fruit segments (each expected to produce one seed) have different dispersal strategies typical of plants with dimorphic fruit (Venable and Brown, 1988). In *C. edentula,* the distal segment disperses away from the maternal plant, and the proximal segment remains attached and is likely buried with the maternal plant after senescence. Different dispersal strategies between fruit halves suggest that variation in maternal resource allocation to distal versus proximal seeds or selective seed abortion may be mechanisms regulating the demographic structure of the next generation (offspring density) (Donohue and Schmitt, 1998). Thus, our greenhouse plants growing in low density (lower competition for resources than high density) and treated with salt spray (nutrient source) were hypothesized to have a higher seed set ratio (fewer proximal seed abortions) than plants in other treatments.

This research addresses the following questions: (1) Do beach plants have higher fitness than dune plants, as was reported by Keddy (1982)? (2) Is there any evidence of genetic variation among plants growing in the beach versus dune zones of the coastal habitat? And, (3) do beach and dune plants respond differently to conditions characteristic of the beach (high sea spray relative to the dune) and dune (high conspecific density relative to the beach) microhabitats?

2.2. METHODS

2.2.1. Study system

The study system for this research was sea rocket, *Cakile edentula* ssp. *edentula* var. *edentula* (Bigelow) Hooker (Brassicaceae). This endemic, coastal plant occurs across the ecological gradient stretching from open sandy beach to densely vegetated dunes along the Atlantic coast from North Carolina, USA to Labrador, Canada, and from the Gulf of the St. Lawrence to the Great Lakes (Rodman, 1974). Additional details describing the beach and dune habitat at each study site are described in Appendix 2.1. *Cakile edentula* is a selfing, annual plant with white to purple coloured petals on small flowers. Each flower produces a dimorphic fruit consisting of a larger distal segment that detaches for dispersal, and a proximal segment that usually remains attached to the maternal plant (Keddy, 1982, Donohue 1997). Although each fruit segment typically produces one seed, the distal segment is more likely to produce a seed than the proximal segment (Keddy, 1982, Donohue 1998a, K.E. Samis *unpublished data*). In Atlantic Canada, seeds germinate and emerge above the sand between May and June, begin flowering in July to August, and senesce in October (Keddy, 1982, B.T. Cole *personal observation*). Plants growing in the beach appear to germinate later, grow to a larger size, and produce more fruit than plants growing in the dune habitat (K.E. Samis *unpublished data*; Keddy, 1982).

2.2.1.1. Definition of plant life stages:

Measured *Cakile edentula* plants were categorized into three life stages; seedling (before reproductive, i.e. no buds or flowers), reproductive, and senescent (flower

production is completed). Senescing plants gradually yellow as a result of the loss of chlorophyll, dehydrate, and disperse distal fruit towards the end of the growing season in late September-October. Plants were surveyed at each developmental stage using bud, flower, and fruit counts to either define life stages of individual plants (for tagged plants and greenhouse experiment plants) or populations, on average (in random plant surveys). The population average life stages for random plants were defined as follows: If fewer than 50% of surveyed plants were reproductive, the population was recorded as in the seedling stage. When more than 50% of the plants were reproductive and fewer than 50% senescent, the population was recorded as reproductive. If more than 50% of the measured plants had fruit but no buds or flowers, the population was recorded as senescent.

2.2.2. Surveys of wild populations and habitat

Empirical population and habitat surveys conducted in summer 2013 provide a description of the potential demographic and ecological correlates of *C. edentula* fitness. Two approaches, random and tagged plant surveys, were used to monitor plant phenotype and ecological environment at a total of nine sites in Nova Scotia (NS), New Brunswick (NB) and Prince Edward Island (PE). The tagged plant surveys (at two of the nine sites) complemented the random surveys by providing comprehensive lifetime data for plants in each zone (beach and dune). However, the random population survey methods were less labour intensive and did not rely on locating previously identified plants, unlike the tagged plant surveys, thus more sites were used for random surveys (seven of the nine sites) to gather general plant and population data. The selection of plant traits measured

for this research was based on previous observations of *C. edentula* in the wild, and current hypotheses on how plants adapt to the coastal habitat.

2.2.2.1. Random population surveys:

The population structure of *C. edentula* was quantified at seven sites (Anglo Rustico, Basin Head, Darnley and Freeland, PE; Taylor's Head and Pomquet, NS; and Kouchibouguac, NB) by measuring plants along a 1 km long strip in each beach and dune zone, running approximately parallel to the water's edge (Appendix 2.1 Figure 1). Plants were randomly selected and were not followed throughout their life cycle. Sampling was conducted using a random number device to generate the number of steps (ranging from 1-20) between each measured plant along a haphazard transect parallel to the water in each zone.

Morphological measurements: Plant size (height x width x length with width measurement taken parallel to the water's edge and length perpendicular to width, cm^3; *size* and *height* included in analyses) and number of lateral branches (those directly attached to primary stem; *branches*) were recorded for 25 randomly chosen plants in the senescence life stage (final survey) per zone. The distance to the closest neighbouring vegetation within a 1 m radius of the surveyed plant (*distance to neighbour*) was also recorded as a proxy for resource competition.

The surveys at senescence included a count of the fruit (whole fruit) produced per plant as an estimate of *fitness*. Data on additional ecological variables (distance to high tide line for each plant, growing substrate [i.e. sand or buried patches of macrophyte wrack – near shore macroalgae and sea grasses], and plant traits [size, and number of

leaves and branches/plant during early life history stages, and bud and flower counts during reproductive stages]) were also recorded for each plant but are not analyzed here.

Fruits were collected during the last survey at Anglo Rustico, Taylor Head, and Kouchibouguac to assess seed set. Approximately 50 whole fruit (distal and proximal) per plant were collected in each zone and stored in paper envelopes to dry. Five distal and five proximal fruit from each plant collection were randomly selected, opened, and seed presence or absence was recorded (*C. edentula* typically produces one seed per fruit half unless a seed was aborted, but 2-3 seeds in a fruit half have been observed; *KES unpublished data*). The ratio of proximal to distal seed produced in five fruits was used to calculate *seed set ratio*.

2.2.2.2. Tagged population surveys:

Individual plants were monitored throughout their life cycle at two sites, Little Harbour, PE, and Martinique, NS (A2.1 Figure 1). Fifty randomly selected plants were tagged in each zone in early June using a labeled flag and a metal tag tied around the primary stem. Plants were chosen and measured (*fitness*, *size*, *height*, *branches*, *distance to neighbour*, and *seed set ratio* [the latter only at Little Harbour]) using the same methods as the random population surveys (Section 2.2.2.1). *Growth rate* for each individual was calculated as the difference between seedling plant size (first size measurement at tagging) from the size at senescence (final size measurement) divided by the number of days between those two measurements. *Flowering duration* for each individual was calculated by subtracting the approximate date of first flower from the approximate date a plant transitioned into senescence (no flowers remain). Sites were visited bi-weekly, starting in June, until plants had senesced and the total fruit count for

all individual plants had been recorded. Since not all plants senesced on the same date, sites were visited twice at the end of the growing season.

Although plants were also initially tagged at Darnley, PE, the data were analyzed as random survey data since the majority of tags were lost within one month of tagging. At that time, random survey methods (Section 2.2.2.1) were employed to compensate for the loss.

During the last survey at Little Harbour, PE in October, approximately 50 whole fruit (distal and proximal) per plant were collected in each zone and stored in paper envelopes to dry and used to calculate seed set ratio (as per Section 2.2.2.1). Fruits were not collected at Martinique, NS due to challenges coordinating survey and collection dates with plant senescence.

2.2.3. Assessing variation in phenology with a reciprocal transplant experiment

A reciprocal transplant experiment between sites and zones within sites was used to comprehensively assess the response of beach and dune plants to abiotic and biotic stressors in native and non-native microhabitats. Reciprocal transplant experiments offer a comprehensive method to measure fitness variation and assess the level of local adaptation among habitats. The experiment was designed to measure the effects of zone, sea spray, sand burial, and density, however due to low survival and complications executing and maintaining the treatments at the transplant sites, only the effect of zone (transplant and maternal plant locations at a site) was assessed. For reference, the methods employed for the initial set-up of the experiment and the excluded experimental treatments are described fully in Appendix 2.2.

2.2.3.1. Transplant experiment design:

Randomly selected fruits (distal and proximal) were collected as per methods outlined in section 2.2.2.1 from wild beach and dune plants in fall 2012 (Table 2.1). All seeds collected from one plant represent one maternal family and are presumed to be full-siblings (maternal plant is the ovule and pollen donor with a primarily selfing mating system; see 2.2.1). Seeds were stored in paper envelopes in the cold and dark, and were scarified (with a scalpel) prior to cold, wet stratification in the dark at ~4 ºC for three days in a refrigerator. After manually removing seed coats, germinated seedlings were planted in plastic plug trays (128 x 2 cm^2 wells) filled with sand collected from the transplant site (Darnley or Martinique) in spring 2013. Trays were placed in a Conviron A1000 germination cabinet for 10 days while emerging, moved to the greenhouse for an additional 12 days, and then hardened at each transplant site for 8-9 days before planting. Seedlings were transplanted in late June into 80 cm x 60 cm experimental transplant blocks with minimal disruption to native vegetation and substrate. Experimental block locations were randomly selected using methods described in section 2.2.2.1. At the Darnley transplant site, experimental plants were initially subjected to one of four planned treatments: control, high-density, blocked sea spray and burial, and at Martinique one of two treatments: control and high-density with ten plots per treatment per zone at each site (see Appendix 2.2 for a full description of the initial design). However, due to low survival (See Results) non-control treatments were discontinued (not included as factors in the final analysis), and all surviving plants were maintained as control plants for the reciprocal transplant analysis. Control plots were planted at low-density with 12 transplants per plot at 20 cm spacing (selected based on previous research of density-

dependent effects; Keddy, 1981); due to low survival across all transplant blocks, all plants included in the final analysis grew at this density or lower. Plants from the same site and zone sources were not placed next to each other to avoid the negative effects of sibling competition, which has been reported in *C. edentula* (Dudley and File, 2007). Experimental plots were spaced at least 5 m apart along a 300 m section running approximately parallel to the shoreline within each zone. In total, 1200 plants were transplanted into 40 experimental plots per zone at Darnley (600 transplants per zone), and 720 plants were transplanted into 20 experimental plots per zone at Martinique (360 transplants per zone).

2.2.3.2. Measurements on experimental transplants:

The survival of experimental transplants was recorded seven days after transplanting to identify mortality related to transplant stress. Date of first flowering was recorded upon first observation of floral buds, and senescence date was recorded once it was observed that a plant was no longer reproductive (no additional buds or flowers produced). *Flowering duration* for each individual was calculated as the difference between the date of first flowers and the date of senescence. The stem length from the cotyledonary node to the apical meristem was recorded at transplanting and at senescence. *Growth rate* was calculated for each individual as the difference between the initial seedling stem length (measured at transplanting) and stem length at senescence (final stem length measurement, represents the variable *stem length*) divided by the number of days between measurements. Finally, total fruit production was recorded at senescence to provide an estimate of *lifetime fitness*. The transplant experiments were completed in September, at which point all experimental plants had senesced.

Data on additional plant traits (stem length, and number of leaves and branches/plant during early life history stages, and bud and flower counts during reproductive stages) were also recorded for each plant, but are not analyzed here.

2.2.4. Assessing plant response to sea spray and density under controlled conditions

A greenhouse experiment was conducted in the UPEI greenhouse (located on the south side of the Duffy Science Center) to assess the response of beach and dune plants to simulated abiotic and biotic stressors representative of conditions occurring in the coastal environment. A greenhouse experiment is a standard approach for assessing plant responses to single or multivariate environmental traits under otherwise standardized conditions. However, given that controlled conditions do not replicate the complexity of interactions occurring in the wild, these data must be interpreted in the context under which they are collected.

2.2.4.1. Greenhouse experimental design:

Sixteen plastic totes (37.9 L; 22.2 cm x 41 cm x 61 cm) with small drainage holes were lined with garden fabric and filled with sand collected in 2013 from the public access Blooming Point dunes, PE (46º24'N, 62º56'W). Seeds collected from Darnley, PE and Martinique, NS in 2012 (Table 2.1) were scarified (with a scalpel) and cold, wet stratified in the dark at ~4 ºC in a refrigerator for three days in petri dishes. After manually removing the seed coats, germinated seeds were pooled by site and zone and sowed into totes at a depth of approximately 0.5 cm, with seeds from the same maternal family not sowed adjacent to each other to avoid sibling competition. Low density totes contained 10 plants/tote (8 totes = 80 plants in the low density treatment) sowed 15 cm

apart in three rows of three or four plants/row. High-density totes contained 18 plants/tote (8 totes = 144 plants in the high density treatment) sowed 6 cm apart in five rows of three or four plants/row.

Greenhouse conditions roughly mimicked late spring day length (14 hr day) with a temperature range of 15-35 ºC for six weeks, followed by a gradual change to fall day length (15 hr day) cycles with a temperature range of 10-30 ºC. Humidity misters in the greenhouse were turned off to permit moisture control through manual watering, which was conducted on a regular basis as necessary to maintain greenhouse plants. Light conditions were monitored in the greenhouse to provide a general measurement of light reaching the greenhouse plants using Photosynthetic Light (PAR) Smart Sensor S-LIA-M003, linked with a HOBO Micro Station Data Logger; Onset, Massachusetts, USA). Totes were rotated weekly to avoid positional affects.

2.2.4.2. Experimental treatments:

Plant responses to two treatments, density and sea spray (also referred to as salt spray), were assessed using a factorial design. The density treatment is described in Section 2.2.4.1. Four totes from each density treatment were subjected to either sea spray or freshwater (tap water from greenhouse hose) spray treatments using standard spray bottles. The sea spray treated plants were misted with seawater collected from Blooming Point, PE in late August 2013 (salinity ~29 ppt; Tang, 2007) and stored at ~4 ºC. The freshwater-sprayed plants were misted with tap water, to eliminate the effect of spray application. Plants were completely coated, including an approximately 2 cm radius of substrate around the plant base, with either spray treatment. Totes were treated

twice/week on a regular schedule for nine weeks starting approximately six weeks after sowing seeds, when the majority of plants (vegetative) had multiple true leaves.

A subset of seeds did not germinate within the first seven days after sowing, and were replaced with seed of the same age (imbibed at the same time as original sewed seeds) and from the same zone and site 10 days after initial sowing.

2.2.4.3. Measurements on experimental greenhouse plants:

The number of budding new leaves was counted on each individual eight times throughout the experiment from the start of treatments to plant senescence. The sum of new leaves for each individual across the eight recordings was divided by the difference between the first and last recording dates of budding leaves to provide an estimate of new leaves produced per day (*new leaves*). Date of first flowering was recorded upon first observation of floral buds, and senescence date was recorded once it was observed that a plant was no longer reproductive (no additional buds or flowers produced). *Flowering duration* was calculated as the difference between date of first flowers and date of senescence (no flowers). Plant *stem length* (cm; cotyledon node to apical meristem), number of lateral branches (those directly attached to primary stem; *total branches*) and primary stem nodes (*total stem nodes*) were recorded at senescence along with the total number of fruit (an estimate of *lifetime fitness*). *Stem growth rate* was calculated for each individual as the difference between stem length at seedling (first time recorded) and stem length at senescence and divided by the difference in days. At maturity, all fruits per plant were collected, dried, and stored in paper envelopes at ~4 ºC in the dark. *Seed set ratio* between distal and proximal fruit was recorded as described in section 2.2.2.1 for five fruit halves per plant. At the end of the experiment, all plants (reproductive or

not) were harvested into paper bags before senescence (at maturity). Above-ground biomass excluding fruit was recorded (to 0.0001 g) before and after drying in a humidity-controlled chamber (Conviron Growth Chamber at ~25 ºC). *Water content* was calculated as the difference between biomass weights divided by the wet weight ([wet weight – dry weight]/ wet weight).

Finally, as potential indicators of plant stress, chlorophyll content and *stem diameter* of each plant were recorded. Low chlorophyll readings may suggest a plant was experiencing higher stress and a reduction in photosynthesis capacity compared to plants with higher chlorophyll readings (Shafi *et al.,* 2011). In addition, stem diameter has been used to estimate plant water status as a stress indicator (Hsiao, 1973). An atLEAF+ chlorophyll meter (atLEAF, FT Green LLC, Delaware, USA) was used on the youngest, fully extended leaf on all plants before the first spray treatment application and mid-experiment (to assess temporal chlorophyll variation throughout the experiment), and on most plants at the first signs of yellowing/senescence. Each reading was replicated 10 times on the same leaf to account for precision error in the positioning of the meter laser. *Mean change in chlorophyll content* (chl) was calculated as the difference between the average of all first measurements recorded (before first treatment) per plant and the average of all final measurements recorded per plant ($chl_{final} - chl_{first}$). Stem diameter (mm; d) was measured immediately below the cotyledon node during and at the end of the experiment using calipers. C*hange in stem diameter* was calculated as the difference between measurements ($d_{end} - d_{during}$). For both measurements, a negative value suggests a decline in plant health, or increased stress.

Data on additional plant traits (stem length, and number of leaves and branches/plant during early life history stages, and bud and flower counts during reproductive stages) were also recorded for each plant but are not analyzed here.

2.2.5. Data analysis

Analysis of variance, linear models and pairwise correlations were used to analyze the data generated in this thesis, and these analyses are described below. All data were analyzed and figures generated using the statistical software "R" (version 3.0.3; R Core Team, 2014). Correlation analyses were conducted with the cor.test function, fixed linear models were conducted using the lm function, and mixed effect linear models were conducted using the lmer function in the lme4 package (Bates *et al.,* 2014) and following the model testing approach described by Zuur *et al.* (2009). Plots demonstrating model results were created using ggplot2 (Wickham, 2009), and standard error bars were calculated in plyr (Wickham, 2011).

Pearson correlation analysis was used to assess the association between measured plant traits from the population surveys (Section 2.2.2) and greenhouse experiment (Section 2.2.4). Correlation assumptions were assessed visually using histograms and scatter plots of the variables included in the pairwise correlation. Log_{10}, square root, or rank transformations were applied to variables to meet model assumptions (Table 2.1). The correlation coefficient and *p*-values are reported for each pairwise correlation model.

Linear models (fixed or mixed; Table 2.2) were used to assess the fixed effects of *zone* (plant location for wild plants and transplant experiment, and seed source location for greenhouse and transplant experiments), *site* (location and/or source population), and

experiment treatment (when applicable; *density* and *spray*) on several quantitative plant traits (fixed and random effects are explained in Table 2.2). For each response variable, outliers (three or more standard deviations from the mean) were removed from the analysis only if their inclusion quantitatively affected the outcome of the model. A hypothesis-testing approach was used to assess the significance of each effect (random effect tests were one-tailed; fixed were two-tailed) by sequentially comparing the full model to reduced models (mixed models used Likelihood ratio chi-square tests; fixed models used F-value tests) without the effect of interest (Zuur *et al*., 2009; West *et al*., 2007). Non-significant, fixed effects were removed from the model. Random effects were retained in the models to keep the same structure for all mixed models (i.e. degrees of freedom), and since these random effects represented biologically meaningful components of the survey/experiment design (Bolker *et al.*, 2008). Chi-squared likelihood (mixed model) or F-values (fixed model), and *P*-values were extracted from each sequential model. Models were validated using normal quantile-quantile plots, residual distributions, and residual versus fitted value plots. Variables were transformed as required to meet linear model assumption. For each variable, means ± standard errors were reported (back-transformed means for Log_{10} and square-root transformed variables, and arithmetic means from non-transformed data for rank transformed variables).

Before applying the mixed model analysis to greenhouse experiment data (Section 2.2.4), the effect of the replant date was assessed using a fixed model ANOVA (analysis of variance; meeting model normality assumptions). If replant date was significant (as it was for analysis on the flowering duration variable; Table 2.2), it was included in the full mixed model (described above) as a fixed effect. If replant date was significant in the

mixed model, the replanted individuals were removed from the model (reduction in sample size from 224 to 168 plants).

2.3. RESULTS

2.3.1. Wild plant population surveys

2.3.1.1. Covariance among wild plant traits

Significant, positive correlations between pairs of plant traits measured in the population surveys (tagged and random) within sites and assessed for beach and dune plants separately (Appendix 2.2 Tables 1-7) were found between the following pairs of traits:

(1) fitness and (a) size (all $P < 0.0067$ in both zones within all sites, except Martinique beach $P = 0.29$), (b) height (all $P < 0.05$; except Martinique beach $P = 0.92$, Anglo Rustico beach $P = 0.053$, Darnley beach $P = 0.092$), and (c) total branches (all $P < 0.025$);

(2) size and (a) height (all $P < 0.0033$; except Martinique beach $P = 0.072$), and (b) total branches (all $P < 0.0013$; except Martinique beach $P = 0.0502$); and

(3) total branches and height (all $P < 0.047$; except Martinique beach $P = 0.602$).

Overall, these associations suggest plants that were larger, grew taller, and had more branches produced more fruit than plants that were smaller and less branchy. All other correlations between traits were non-significant.

2.3.1.2. Assessing zone effect on wild plant traits

Mixed, linear model analysis of wild plant traits (Table 2.3) revealed significant variation between zones in fitness ($P = 0.0021$), size ($P = 0.0022$), height ($P = 0.017$), number of branches ($P = 0.004$), and distance to the nearest neighbour ($P = 0.0012$). For all traits, the random effects that allowed for trait variation among sites (all $P < 0.0001$) and variation between zones across sites (slope; all $P < 0.0035$) were significant. These data reveal that at the majority of sites, beach plants produced more fruit (Figure 2.2A), were larger (Figure 2.2B) and taller, had more branches, and were further away from their neighbours than dune plants (summary statistics per zone and trait presented in Table 2.4).

Seed-set ratio (proximal to distal seeds per flower) of wild plants did not vary significantly between beach and dune plants (main effect of zone $P = 0.36$; Tables 2.3, 2.4) growing at Anglo Rustico, Kouchibouguac, Little Harbour, or Taylor Head. In addition, no significant variation in growth rate ($P = 0.20$) or flowering duration ($P = 0.58$) was detected between wild beach and dune plants followed throughout their life cycle at Little Harbour or Martinique (tagged plants; Tables 2.3, 2.4). However, despite the lack of an overall difference between zones, the slope and direction of change in seed-set ratio and growth rate between zones varied significantly among sites for both traits (variation between zones among sites: seed set ratio $P = 0.028$, growth rate $P = 0.0001$).

2.3.2. Transplant experiment

Due to vandalism shortly after setting up the experimental treatments, and possibly high post-transplant stress, early survival in this experiment was low. Only 3% of Darnley transplants (36 of 1200 plants) survived to flower and of those only 24 plants survived to produce fruit. Only 5% of Martinique transplants (36 of 720 plants) survived

to flower and of those only 28 plants survived to produce fruit (Table 2.5; original sample sizes of 600/zone at Darnley, and 360/zone at Martinique). For that reason, the blocked sea spray, sand burial, and high-density treatments were removed from this experiment, leaving the assessment of zone effect on experimental plant fitness. Furthermore, the loss of transplants meant the blocking effect was also lost because so few plants remained per experimental plot (reduced to range 0-4 from the initial 12 or 24 plants per plot). For that reason, and to retain degrees of freedom the block effect was dropped from statistical models of these data (Table 2.2).

In addition, Darnley beach transplants were excluded from analysis since only two out of 30 plants that survived transplanting into the beach at Darnley also survived to produce fruit. The survival to senescence from the number that survived transplanting in other transplant zones within sites was as follows: Darnley dune had 17 from 30 surviving transplants, Martinique beach had 15 from 27 surviving transplants, and Martinique dune had 12 from 30 surviving transplants survive to produce fruit.

Given that the sample sizes did not allow for zone comparisons at Darnley, the variation between Darnley dune transplants and Martinique dune transplants was assessed instead. This analysis revealed significant variation between sites in growth rate of transplants in the dune (between Darnley and Martinique site effect for dune transplants $P = 0.0055$), but no significant variation in lifetime fitness ($P = 0.15$; Tables 2.5B, 2.6B), stem length ($P = 0.53$), or flowering duration ($P = 0.50$). These results suggest Darnley and Martinique dune transplants were fairly similar aside from growth rate, and data collected from one site may be used to infer variation at the other.

The research objective of assessing zone variation was analyzed in a second analysis using only data from the Martinique transplant site. At Martinique, lifetime fitness varied significantly among beach and dune transplants (transplant zone effect P = 0.00046) and occurred in the same direction as detected in the empirical surveys of wild plants; that is, on average, beach transplant fitness was higher than dune transplant fitness (Table 2.5A, 2.6A; Figure 2.3A). Variation between Martinique transplant zones for other quantitative traits was weak (growth rate, $P = 0.042$; Figure 2.3B) to non-significant (stem length $P = 0.073$, flowering duration $P = 0.61$; Table 2.5A, 2.6A), but the trends generally supported results from empirical surveys (reported in Table 2.3) suggesting beach plants consistently grew faster and larger, and had a longer flowering duration than dune plants.

Taken together, these data provide some support for patterns of variation observed in the wild, particularly that beach plants have higher fitness than dune plants, but this hypothesis may be complicated by low survival in the transplant experiment.

2.3.3. Greenhouse experiment

2.3.3.1. Covariance among plant traits

Lifetime fitness in greenhouse plants showed a positive and significant correlation with stem length ($P = 0.00048$), total branches ($P < 0.0001$), and flowering duration ($P < 0.0001$; Table 2.7). Overall, plants that were taller, had more branches, and flowered longer had higher fitness than smaller, less branchy plants with a short flowering duration. Stem length *(*$P = 0.039$) and total branches ($P < 0.0001$) were significantly correlated with the production of new leaves, with short and branchy plants having the

highest leaf turnover. The total number of branches also increased significantly with flowering duration ($P = 0.018$). All other pairwise correlations revealed non-significant associations between assessed traits (Table 2.7).

2.3.3.2. Assessing the effect of greenhouse treatment and source zone on plant traits

All measured plant traits significantly varied between at least one of the experimental treatments (Table 2.8). Overall, salt spray appeared to have the most frequent association with variation in plant traits, but there was also evidence of density-dependence and variation between source zones for some traits. Significant two-way treatment interactions were detected in only four of the 11 traits. Three-way interactions between source zone, salt spray and density treatments were not significant in any mixed model analyses (all $P > 0.13$).

Analysis of the effect of zone, spray, and density on plant traits (Table 2.8) revealed a significant interaction between spray treatment and density on lifetime fitness ($P = 0.0083$; Figure 2.4A) and the number of branches ($P < 0.0001$; Figure 2.4B). Multiple comparisons (details of Tukey's HSD tests in A2.3 Table 1, and summarized here and in Table 2.9) revealed that freshwater-sprayed plant fitness and number of branches ($P = 0.35$) did not vary between densities. However, salt-sprayed plants in high density had significantly lower fitness and fewer branches ($P = 0.0013$) than plants in low density (Table 2.9). Also, there was marginally non-significant evidence of genetically based variation in fitness (source zone effect $P = 0.087$) and the number of branches per plant ($P = 0.082$) between beach- and dune-sourced plants. All other effects on fitness and branches were non-significant (all $P > 0.108$) in both models (Table 2.8).

The effect of spray treatment on stem growth rate varied significantly between plants from each zone source (zone x spray $P = 0.022$; Table 2.8; Figure 2.5A), which may be evidence of genetic variation, and between source sites ($P = 0.034$; not shown in Figure 2.5). Stem growth rate of beach sourced plants from both source sites was significantly higher in the freshwater spray treatment than in the saltwater treatment (zone x spray x site Table 2.9). Dune plant growth rate was also higher with freshwater treatment than with salt spray treatment for plants originating from seed collected at Darnley, but the difference between treatments was marginally non-significant for plants originating from dune seed collected at Martinique ($P = 0.084$), although trending in the same direction. Overall, high density plants had a significantly lower growth rate than plants in low density ($P = 0.00081$; density effect Table 2.9). However, the effect of density on growth rate showed weak, but non-significant variation between plants grown from beach and dune-collected seeds (zone x density $P = 0.088$), but more data will be required to determine if this interaction is real.

The effect of density on proximal to distal seed set ratio varied marginally by source zones ($P = 0.046$; Table 2.8; Figure 2.6; zone and density interaction Table 2.9). However, Tukey's comparisons between zones by density were non-significant (Table 2.9), suggesting further investigation into seed set ratio, and possibly higher sample sizes are required to determine the biological relevance of potential variation in this trait.

Primary stem length (at senescence) varied significantly between zones ($P = 0.0009$), and between spray ($P = 0.0006$) and density treatments ($P = 0.023$; Figure 2.5B). Although none of the interactions among treatments were significant (Table 2.8), variation among density and spray treatments (density x site $P = 0.075$) occurred in the

same direction as detected for growth rate (Fig 2.5). Beach plants had a longer stem at fruiting than dune plants (zone effect, Table 2.9), and salt-sprayed plants had a shorter stem than freshwater-sprayed plants (spray effect, Table 2.9). Also, plants growing in high density were shorter than plants in low density (density effect, Table 2.9).

Zone was the only factor significantly affecting total number of nodes on the primary stem ($P = 0.0088$; Table 2.8) and flowering duration ($P = 0.03$). Beach plants produced more nodes along the primary stem than dune plants (zone effect, Table 2.9) regardless of source site, spray or density treatment. Although there was a significant effect of source site (random) in the model ($P = 0.018$), beach plants from both source sites ($P = 0.035$) had longer flowering durations than dune plants (zone x site, Table 2.9).

In contrast to all other traits, except seed ratio, the remaining traits (new leaves, change in stem diameter, water content and mean change in chlorophyll content) did not vary among plants by source zone (all $P > 0.22$). In addition, there were no detectable interaction effects for any of these traits (Table 2.8). However, variation in all four traits varied significantly by spray treatment: number of new leaves produced by a plant per day ($P = 0.0001$), change in stem diameter ($P = 0.0007$), plant water content ($P < 0.0001$), and mean change in chlorophyll content of leaves ($P < 0.0001$). The effect of plant density was significant, albeit only marginally so, for mean change in chlorophyll ($P = 0.046$; Table 2.8), but considerably less influential on the other three traits (new leaves, $P = 0.077$; stem diameter, $P = 0.071$; and water content $P = 0.89$). For three traits there was also significant variation among plants originating from seed collected at the two sites (new leaves $P = 0.0049$, stem diameter $P = 0.0072$, and water content $P < 0.0001$; Table 2.8). Darnley- and Martinique-sourced plants sprayed with freshwater

produced fewer leaves per day, displayed a reduced change in stem diameter, and increased water content compared to those sprayed with saltwater (spray x site, Table 2.9).

2.4. DISCUSSION

Through empirical and experiment analysis, this research has confirmed that fitness of *Cakile edentula* plants growing naturally on the beach is significantly greater than plants growing in the dune. However, in the greenhouse experiment, plant fitness was not dependent on the maternal environment. These data suggest that plastic variation in plant traits in response to the local growing environment is an important factor determining fitness in this species. Experimental investigation in the greenhouse of the effects of specific coastal factors demonstrated that growing density and salt spray exposure had significant effects on fitness regardless of the maternal plant origin. In contrast, some quantitative traits associated with fitness, including flowering duration and size related traits, were influenced by the maternal environment (source zone), but this relationship sometimes varied with density and spray treatments, as was the case with stem growth rate. In all cases, as observed in the field, the zone effect suggested that the offspring from beach plants would have higher growth than offspring from dune plants. These data suggest that the effects of environment on fitness are complex and likely occur through interactions with several biotic and abiotic factors. Overall, this research provided the opportunity to assess phenotypic variation among beach and dune plants and

their offspring, and provides insight on the genetic basis and plasticity of traits associated with reproduction and survival in the coastal habitat.

2.4.1. Trait associations with fitness

Empirical *Cakile edentula* wild plant surveys and a greenhouse experiment confirmed observations by Keddy (1982), and Donohue (1998b), that large *C. edentula* plants with more lateral growth accommodate the production of more fruit. These size and branch associations with plant fitness were found in the majority of wild beach and dune plant population surveys conducted over the 2013 growing season and in the greenhouse experiment. Additionally, a longer flowering duration in greenhouse plants (trait associations with flowering duration of transplants not assessed due to low survival) was associated with higher fruit production, as might be expected when more time and resources are allocated to the reproductive phase of development. The timing and length of the reproductive period in semelparous plants (i.e. plants that senesce after a single reproductive event, including annuals like *C. edentula*) influences the quality and number of offspring produced (Hughes and Simons, 2014). Flowering duration may also indirectly affect fitness through an environmental variable present during the extended flowering period. This may lead to variation in reproductive efforts if the effect of the environmental variable changes over time or is not experienced by all reproductive plants, such is the case when early versus late flowering phenotypes reproduce under different environmental conditions during the growing season (Giménez-Benavides *et al.* 2010). Further investigation to determine traits directly associated with *C. edentula* fitness within and between habitats is required and could involve additional transplant

experiments, ideally with comprehensive ecological monitoring tied to each plant's developmental and life stage transitions.

2.4.2. Plant response to environmental factors

Salinity, specifically from sea spray, is believed to be one of the primary abiotic factors defining zonation in the coastal environment, and for this reason salt tolerance in coastal plants has been well studied (e.g. Rozema *et al.,* 1982; Boyd and Barbour, 1986; Debez *et al.,* 2004). The general consensus is that beach plants are under higher salt stress than dune plants, but the effect of salinity varies by species (e.g. Sykes and Wilson, 1988). Plants of the genus *Cakile* are a model system for assessing salinity tolerance because they grow along a salinity gradient from beach to dune, as well as in marine and lacustrine habitats (*C. maritima* [European sea rocket] and *C. edentula*: Boyd and Barbour, 1986; Debez *et al.,* 2012; Debez *et al.,* 2013). Moreover, salinity trials have suggested the presence of specific salt tolerance physiological mechanisms in *C. maritima* (Megdiche *et al.,* 2008), which may suggest that adaptations to salt tolerance are present in all coastal endemics in the *Cakile* genus.

In my greenhouse experiment assessing *Cakile edentula* plant response to salt spray and density, the salt spray treatment had the greatest impact on plant traits, with more phenotypic differences observed between seawater and freshwater-sprayed plants than between growing densities or seed sources. Increased succulence and leaf turnover are common plastic plant responses for tolerating salt (Maun, 2009). However, while increased leaf turnover in greenhouse plants was associated with salt spray treatment compared to freshwater treatment, succulence at maturity (before complete senescence)

showed the opposite effect with water content reduced in the salt spray treatment compared to the fresh water treatment. It has been speculated that there is a peak succulence response to salt in some plants (Parida and Das, 2005; e.g. Khan *et al.,*1999), and that water and osmotic potential are negatively affected by salt water while turgor pressure increases with salinity (Parida and Das, 2005). These data may suggest there is an optimal time to measure succulence, which may have been missed in this experiment when recorded only at plant maturity.

Another indicator of potential plant stress is reduced chlorophyll content. Specifically, chlorophyll activity is expected to decrease in salt-stressed plants because salt ions may inhibit the biosynthesis of chlorophyll (Ali *et al.,* 2004). Previous greenhouse experiments on *C. maritima*, a close relative of *C. edentula* with a similar coastal niche, did not reveal significant changes in plant chlorophyll content up to 500 mM NaCl (~27 ppt), however there was a tendency for reductions in chlorophyll content relative to controls (Debez, 2006). Chlorophyll levels in our greenhouse grown *C. edentula* plants were lowest when sprayed with seawater (~29 ppt, typical seawater salinity in the Gulf of St. Lawrence; St. Lawrence Global Observatory-SLGO, 2003) compared to plants sprayed with tap water. Although Debez *et al.* (2004, 2006, 2012, 2013) did not assess *C. maritima* chlorophyll response in treatments above 500 mM of salts (lower than typical salt concentration in the Gulf of St. Lawrence), the direction of chlorophyll content change in the two species is qualitatively similar. These data suggest that natural seawater salinity at our study sites may not be optimal for *C. edentula* growth, and may suggest salinity exposure was of higher concentration and/or more frequent in the experiment than occurs in the wild. The change in chlorophyll levels was

also negatively associated with high experimental plant growing density in the greenhouse. Other studies in a natural setting have attributed reduced chlorophyll levels with high competition to limited resources and plant dehydration (e.g. *Pinus taeda L.,* [Pinaeceae, Loblolly pine] Carter *et al.,* 1989). While the environmental association remains unclear, in *C. edentula*, plant stem length and the change in chlorophyll levels were reduced by salt spray compared to the freshwater treatment as expected. Chlorophyll content was also not measured on plants in the natural environment and although this might have been logistically more difficult due to variation in weather, at this point it should be kept in mind that the data analyses presented here may only be relevant to the controlled environment.

While salt exposure may have contributed to greater phenotypic variation than growing density among plants in the greenhouse (e.g. Table 2.8), variation in two plant traits was significantly associated with density. Plant stem elongation and growth rate were significantly reduced by high conspecific competition compared to when competition was low (regardless of source zone). Resource limitations are often assumed to occur when individuals are growing in high density, and may result in stunted plant growth. However, Dudley and Schmitt (1996) reported that stem elongation in *Impatiens capensis* (Balsaminaceae) plants was associated with increased fitness when growing in high density because stem elongation reduced the negative effects of shading. However, stem elongation reduced fitness when plants were growing in low density, and this was hypothesized to be due to the costly use of resources when elongation was not beneficial. While Dudley and Schmitt (1996) directly assessed plant fitness under vegetation shading and my experiment indirectly assessed above and below ground competition, the effects

of high plant growing density clearly has complex and variable results among plant species and environments.

Saltwater-sprayed *C. edentula* plants growing in low density had a higher mean fitness and produced more branches than plants in any other spray-density treatment (Fig 2.4 and 2.5). However, given that fitness in this plant has been described as density-dependent (Keddy, 1981), it is not surprising that the additional stress of high growing density on salt-sprayed plants was associated with reduced fitness compared to plants growing in low density. Moreover, one may infer from these data that the negative effects of high density outweigh the positive effects of salt spray. Although coastal plants have adaptive mechanisms for dealing with salinity, the differences in plant phenotypes between freshwater- and saltwater-sprayed plants in the greenhouse experiment suggest that *C. edentula* responds plastically to salt spray, and that this response may be associated with the local (above or below-ground) environment of each plant. Given the variation in the combined effect of sea spray and growing density on greenhouse plant traits, it is hard to conclude the overall relationship (e.g. additive, synergistic, etc…) of the two factors.

2.4.3. Effect of zone on plant traits

The results of wild plant surveys and the transplant experiment presented here confirmed observations by Keddy (1982) that fitness (total fruit production) is greater for plants growing on the beach than in the dune. However, estimated fitness of transplant and greenhouse experiment plants was not dependent on the beach or dune source, indicating fitness may be the result of direct plant interactions with the environment.

Similarly, a study by Hereford and Winn (2008) suggested the pattern of *Diodia teres* (Rubiaceae, another selfing annual) plant fitness among six populations from different habitat types weakly supported a hypothesis of local adaptation. However, they did not find evidence of adaptive divergence between populations occurring in the same habitat; that is, local adaptation was not common overall, but was more apparent between populations from different habitats than more similar habitats. Applying the results of the Hereford and Winn (2008) study to our research may suggest that the beach and dune habitats are not strongly contrasting environments and therefore natural selection is not likely to produce a pattern of local adaptation. This hypothesis would further suggest that seed recruitment between the beach and dune habitats would not be constrained by maladapted alleles migrating from one habitat zone to another.

Greenhouse plant fitness varied with spray treatment and plant density, which are environmental factors characteristic of beach and dune habitat. While Keddy (1981) suggested that *C. edentula* fitness was density-dependent, Keddy also hypothesized that density-dependence varied with the growing location, or between beaches and dunes. Since we know that salt spray deposition declines from beach to dune, and that natural plant density is highest in the dune, it could be hypothesized that the effect of density on fitness may depend on interactions with sea spray along the gradient. The greenhouse experiment supports this assertion with salt-sprayed plants in low density having higher fitness than other treatments, and salt-sprayed plants in high density having the lowest fitness.

In agreement with observations by Keddy (1982) and Rodman (1974), beach-sourced plants had a higher growth rate than dune-sourced plants in the greenhouse

experiment, but these results varied with spray treatment. As also reported in a large study on forbs (*Solidago puberula* [Asteraceae], *Solidago rugosa* [Asteraceae], and *Gaylussacia baccata* [Ericaceae]) and trees (*Pinus rigida* [Pinaceae] and *Quercus ilicifolia* [Fagaceae]) native to coastal sand plains (Griffiths and Orians, 2003), salt spray reduced the growth rate of beach and dune plants (plants grown from seed collected in those zones) in our greenhouse experiment. In addition, the expected difference between beach and dune plant growth rate was still detectable after including the salt spray effect. The transplant experiment also revealed higher growth rate for plants growing in beach than for those growing in the dune. While these results suggest important habitat effects on plant phenotypes, there was little evidence of a genetic basis to growth rate to the beach versus dune environment.

As reported in *Abutilon theophrasti* (Malvaceae; velvetleaf, a selfing annual weed) plants growing in cornfields, plasticity in stem elongation can quickly have negative consequences when competition with taller plants for light is impossible to overcome (Weinig, 2000). In the case of *C. edentula*, adaptation to reduced light levels and maintaining a favourable height seems beneficial when competing with tall dune grasses in an effort to avoid wasting resources. Wild *C. edentula* plant surveys suggested beach plants were typically larger and taller than dune plants, a finding that is in agreement with previous observations in *C. edentula* (Keddy, 1982). Although marginally non-significant (low populations numbers from high mortality), the transplant experiment showed the same trend in plant height between growing zones. Observable size differences in wild beach and dune plants also continued into the F1 generation plants growing under uniform greenhouse conditions, suggesting beach and dune plant stem elongation has a

genetic basis and is not strongly plastic, but whether or not this trait is adaptive to each habitat zone needs to be assessed further in a reciprocal transplant experiment.

Donohue (1998a) suggested *C. edentula* ssp. *edentula* var. *lacustris* flowers abort proximal seeds when growing in unfavourable conditions, thereby reducing the at-home seed shadow and increasing the investment into dispersing propagules. It seems likely that this strategy increases the potential for dispersing offspring to potentially higher quality habitat away from home. Fitness and growth observations in wild populations suggest that the dune may be a lower quality habitat than the beach. Since high-density patches of *C. edentula* are also typically observed in the dune, it seems likely that Donohue's (1998a) hypothesis for maternal resource allocation effects on seed production should result in more proximal than distal seed abortions in dune plants and/or for plants growing in high density. Data from tagged wild plant surveys support this assertion and revealed plants at two of the four sites produced a lower seed set ratio (i.e. more distal than proximal seed production) in the dune than on the beach (although overall the pattern is elusive with the other two sites showing a higher or similar ratio in the dunes compared to the beach). A transplant experiment with *C. edentula* at Darnley, PE in 2012 also supported greater proximal seed abortions (KES *unpublished data*). However, the relationship observed in my greenhouse experiment was more complex, with beach- and dune-sourced plants having opposite seed set responses to high and low density. Specifically, beach sourced plants produced more equal seed set between when grown in high density than low density, while dune sourced plants produced more equal seed set between fruit types when plants were grown in low density than high density. These apparent density dependent differences in seed set between beach and dune

sourced plants in greenhouse F1 generations, may indicate plants have genetically-based, instead of maternally-influenced, seed abortion rates. However, another generation of data collected in the greenhouse, where all maternal plants produce seed under the same conditions, is required to adequately tease apart the effects of environment vs. genetics. That being said, other greenhouse experiments with *C. edentula* measuring growth and fitness responses to varying substrate conditions revealed fairly consistent growth trends within families for first- and second-generation plants (J.A. Butler and K.E. Samis *unpublished data*), suggesting maternal effects may not be as influential in F1 generations as often expected. Overall, the main focus of this study was not to determine how plants adapt to the coastal environment, but instead to assess phenotypic and fitness variation between beach and dune, with an additional interest in the adaptive mechanism behind the phenotypic response to common coastal conditions.

Although the seed set ratio between distal and proximal fruits is lower in dune plants than beach plants, the differences in our estimate of fitness (fruit set) would still occur since dune plants clearly produce fewer fruit (and therefore fewer seeds) than beach plants.

2.5. CONCLUSION

The results of this study revealed several phenotypic and fitness differences between beach and dune *Cakile edentula* plants. While traits noticeably varied between plants growing on the beach compared to those growing in the dune, only a few phenotypic differences were suggested as genetically based. That being said, evidence for

plasticity in plants in the literature is quite robust, and the results of this study support the strong ability of coastal plants to respond plastically to their dynamic environment.

Dune habitat is often described as a relatively poor quality habitat. In agreement with that description, this research suggested *C. edentula* has lower fitness and is shorter when growing in the dune compared to in the beach. The ultimate causes of this reduction in habitat quality have only been hypothesized, however the greenhouse experiment carried out for this thesis indicated high conspecific density, a biotic influence on *C. edentula* typically characteristic of the dune habitat, may strongly influence fitness (with the interaction of salt spray) and phenotype variation between beach and dune. While this study only able to assess conspecific density effects under controlled conditions, it seems likely that the characteristic dune grasses will also have a high impact on surrounding species' survival and reproductive output.

Salt spray also had a strong impact on *C. edentula* plant phenotype. Typical stress responses to salinity, including high leaf turnover, were apparent in plants sprayed with seawater in the greenhouse experiment, and salt spray also reduced plant height. However, plant fitness was significantly higher in the salt spray treatment when the growing density was low. Sea spray likely defines coastal zonation, but through interactions with other abiotic and biotic factors.

High growing density negatively affects plant growth, likely due to competition for resources. While it was assumed that dune sourced plants growing in high density would perform better than beach sourced plants (indicating local adaptation to the microhabitat), this was not the case. High growing density in *C. edentula* may be typical of the dune, but it is speculated that seed banks developed from source-sink dispersal provide a

substantial supplement to the native dune seed bank and may lead to these abundance patterns.

In conclusion, coastal plants are highly plastic, and fitness and most phenotypic differences between beach and dune are likely due to the effects of habitat quality. Conspecific competition in the dunes is apparent in *C. edentula*, and given the density-dependence of fitness, growing density may be one of the main factors defining *C. edentula* distribution in the dune. The sea spray gradient seems to coincide with vegetation zonation in coastal habitat. Coastal plants are adapted to tolerate salinity, however *C. edentula* and other species typical of the beach-foredune habitat likely respond plastically to sea spray.

Future studies should repeat transplant experiments along the coastal gradient and between populations to validate the mechanisms of adaptation in coastal plants for the general purpose of predicting coastal vegetation response to impending climate and habitat changes. In addition, further investigation of other biotic and abiotic factors influencing the current distribution of coastal vegetation would be valuable to our understanding of coastal environment stressors.

LITERATURE CITED

Ali Y, Aslam Z, Ashraf MY, Tahir GR. 2004. Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment. *International Journal of Environmental Science and Technology*, 1:221–225.

Alokam S, Chinnappa CC, Reid DM. 2002. Red/far-red light mediated stem elongation and anthocyanin accumulation in *Stellaria longipes*: differential response of alpine and prairie ecotypes. *Canadian Journal of Botany*, 80:72–81.

Angert AL. 2009. The niche, limits to species' distributions, and spatiotemporal variation in demography across the elevation ranges of two monkeyflowers. *Proceedings of the National Academy of Science*, 106 (Suppl 2):19693–19698.

Antonovics J. 1976. The Nature of Limits to Natural Selection. *Annals of the Missouri Botanical Garden,* 63:224–247.

Barbour MG. 1970. Germination and early growth of the strand plant *Cakile maritima*. *Bulletin of the Torrey Botanical Club,* 97:13–22.

Barbour MG. 1978. Salt spray as a microenvironmental factor in the distribution of beach plants at Point Reyes, California. *Oecologia,* 32:213–224.

Bates D, Maechler M, Bolker B, Walker S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-6. http://CRAN.R-project.org/package=lme4.

Baythavong BS. 2011. Linking the spatial scale of environmental variation and the evolution of phenotypic plasticity: selection favors adaptive plasticity in fine-grained environments. *American Naturalist*, 178:75–87.

Bhatt MV, Khandelwal A, Dudley SA. 2011. Kin recognition, not competitive interactions, predicts root allocation in young *Cakile edentula* seedling pairs. *New Phytologist*, 189:1135–1142.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JS. 2008. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution*, 24:127–135.

Boyce SG. 1951. Salt hypertrophy in succulent dune plants. *Science,* 114: 544–545.

Boyd RS, Barbour MG. 1986. Relative salt tolerance of *Cakile edentula* (Brassicaceae) from lacustrine and marine beaches. *American Journal of Botany*, 73:236–241.

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, 13:115–155.

Bridle JR, Polechová J, Kawata M, Butlin RK. 2010. Why is adaptation prevented at ecological margins? New insights from individual-based simulations. *Ecology Letters,* 13:485–494.

Bridle JR, Vines TH. 2007. Limits to evolution at range margins: when and why does adaptation fail? *Trends in Ecology and Evolution,* 22:140–147.

Brown J, Colling A, Park D, Philips J, Rothery D, and Wright J. 1989. Seawater: its Composition, Properties and Behaviour. Pergamon Press, Oxford.

Brown JH. 1984. On the relationship between abundance and distribution of species. *American Naturalist,* 124:255–279.

Callaway RM, Pennings SC, Richards CL. 2003. Phenotypic plasticity and interactions among plants. *Ecology,* 84:1115–1128.

Carter GA, Paliwal K, Pathre U, Green TH, Mitchell RJ, Gjerstad DH.1989. Effect of competition and leaf age on visible and infrared reflectance in pine foliage. *Plant, Cell & Environment,* 12:309–315.

Channell R, Lomolino MV. 2000. Trajectories to extinction: spatial dynamics of the contraction of geographical ranges. *Journal of Biogeography*, 27:169–179.

Cumming GS. 2002. Comparing climate and vegetation as limiting factors for species ranges of African ticks. *Ecology,* 83:255–268.

Davidson-Arnott RGD, Law MN. 1990. Seasonal patterns and controls on sediment supply to coastal foredunes, Long Point, Lake Erie. KF Nordstrom, NP Psuty, and RWG Carter (eds.), Coastal Dunes: Forms and Process, Wiley, Chichester, UK.

Debez A, Ben Rejeb K, Ghars MA, Gandour M, Megdiche W, Ben Hamed K, Ben Amor N, Brown SC, Savouré A, Abdelly C. 2013. Ecophysiological and genomic analysis of salt tolerance of *Cakile maritima*. *Environmental and Experimental Botany,* 92:64–72.

Debez A, Braun H-P, Pich A, Taamalli W, Koyro H-W, Abdelly C, Huchzermeyer B. 2012. Proteomic and physiological responses of the halophyte *Cakile maritima* to moderate salinity at the germinative and vegetative stages. *Journal of Proteomics,* 75:5667–5694.

Debez A, Hamed KB, Grignon C, Abdelly C. 2004. Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritima*. *Plant and Soil*, 262:179–189.

Debez A, Saadaoui D, Ramani B, Ouerghi Z, Koyro H-W, Huchzermeyer B, Abdelly C. 2006. Leaf H+-ATPase activity and photosynthetic capacity of *Cakile maritima* under increasing salinity. *Environmental and Experimental Botany,* 57:285–295.

Dlugosch KM, Hays CG. 2008. Genotypes on the move: some things old and some things new shape the genetics of colonization during species invasions. *Molecular Ecology,* 17:4583–4585.

Donohue, K. 1997. Seed dispersal in *Cakile edentula* var. *lacustris*: decoupling the fitness effects of density and distance from home site. *Oecologia,* 110:520–527.

Donohue K. 1998a. Effects of inbreeding on traits that influence dispersal and progeny density in *Cakile edentula* var. *lacustris* (Brassicaceae). *American Journal of Botany*, 85:661–668.

Donohue K. 1998b. Maternal determinants of seed dispersal in *Cakile edentula*: fruit, plant, and site traits. *Ecology,* 79:2771–2788.

Donohue K. 1999. Seed dispersal as a maternally influenced character: Mechanistic basis of maternal effects and selection on maternal characters in an annual plant. *American Naturalist*, 154:674–689.

Donohue K. 2005. Niche construction through phenological plasticity: life history dynamics and ecological consequences. *New Phytologist*, 166:83–92.

Donohue K. 2009. Completing the cycle: maternal effects as the missing link in plant life histories. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 364:1059–1074.

Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J. 2005. Niche construction through germination cueing: life-history responses to timing of germination in *Arabidopsis thaliana*. *Evolution,* 59:771–785.

Donohue K, Schmitt J. 1998. Maternal environmental effects in plants: adaptive plasticity? 137–158 *in* TA Mousseau and CW Fox (eds.) Maternal Effects as Adaptations. Oxford University Press, New York.

Dorn LA, Pyle EH, Schmitt J. 2000. Plasticity to light cues and resources in Arabidopsis *thaliana*: Testing for adaptive value and costs. *Evolution,* 54:1982–1994.

Dudley SA. 1996. The response to differing selection on plant physiological traits: evidence for local adaptation. *Evolution*, 50:103–110.

Dudley SA, File AL. 2007. Kin recognition in an annual plant. *Biology Letters*, 3:435–438.

Dudley SA, Schmitt J. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *American Naturalist*, 147:445–465.

Eckert CG, Samis KE, Lougheed SC. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology,* 17:1170–1188.

Ellouzi H, Hamed KB, Cela J, Munné-Bosch S, Abdelly C. 2011. Early effects of salt stress on the physiological and oxidative status of *Cakile maritima* (halophyte) and *Arabidopsis thaliana* (glycophyte). *Physiologia Plantarum,* 142:128–143.

Forey E, Lortie CJ, Michalet R. 2009. Spatial patterns of association at local and regional scales in coastal sand dune communities. *Journal of Vegetation Science*, 20:916–925.

Gaston KJ. 2003. The Structure and Dynamics of Geographic Ranges. Oxford University Press, Oxford.

Gaston KJ. 2009. Geographic range limits: achieving synthesis. *Proceedings of the Royal Society B: Biological Sciences,* 276:1395–1406.

Geber MA. 2008. To the edge: studies of species' range limits. *New Phytologist*, 178:228–230.

Geber MA. 2011. Ecological and evolutionary limits to species geographic ranges. *American Naturalist,* 178:S1–S5.

Ghalambor CK, McKay JK, Carroll SP. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, 21:394–407.

Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J. 2008. Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology*, 17:167–178.

Gilman SE. 2006. The northern geographic range limit of the intertidal limpet *Collisella scabra*: a test of performance, recruitment, and temperature hypotheses. *Ecography,* 29:709–720.

Giménez-Benavides L, García-Camacho R, Iriondo JM, Escudero A. 2010. Selection on flowering time in Mediterranean high-mountain plants under global warming. *Evolutionary Ecology*, 25:777–794.

Gómez JM, Valladares F, Puerta Piñero C. 2004. Differences between structural and functional environmental heterogeneity caused by seed dispersal. *Functional Ecology,* 18:787–792.

Gomulkiewicz R, Kirkpatrick M. 1992. Quantitative genetics and the evolution of reaction norms. *Evolution,* 46:390–411.

Gooding EGB. 1947. Observations on the sand dunes of Barbados, British West Indies. *Journal of Ecology*, 34:111–125.

Griffiths ME, Orians CM. 2003. Salt spray differentially affects water status, necrosis, and growth in coastal sandplain heathland species. *American Journal of Botany*, 90:1188–1196.

Guo Q, Taper M, Schoenberger M, Brandle J. 2005. Spatial-temporal population dynamics across species range: from centre to margin. *Oikos,* 108:47–57.

Hall MC, Willis JH. 2006. Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. *Evolution*, 60:2466–2477.

Hargreaves AL, Eckert CG. 2014. Evolution of dispersal and mating systems along geographic gradients: implications for shifting ranges. *Functional Ecology,* 28:5–21.

Hargreaves AL, Samis KE, Eckert CG. 2014. Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. *American Naturalist*, 183:157–173.

Hereford J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *The American Naturalist*, 173:579–588.

Hereford J, Winn AA. 2008. Limits to local adaptation in six populations of the annual plant *Diodia teres*. *New Phytologist,* 178:888–896.

Hesp P. 2002. Foredunes and blowouts: initiation, geomorphology and dynamics. *Geomorphology,* 48:245–268.

Hoffmann AA, and Blows MW. 1994. Species borders: ecological and evolutionary perspectives. *Trends in Ecology and Evolution*, 9: 223–227.

Holdren CE, Ehrlich PR. 1981. Long range dispersal in checkerspot butterflies: Transplant experiments with *Euphydryas gillettii*. *Oecologia,* 50:125–129.

Holt RD, Barfield M. 2011. Theoretical perspectives on the statics and dynamics of species' borders in patchy environments. *American Naturalist*, 178:S6–S25.

Holt RD. 2003. On the evolutionary ecology of species' ranges. *Evolutionary Ecology Research*, 5:159–178.

Holt RD, Gaines MS. 1992. Analysis of adaptation in heterogeneous landscapes: Implications for the evolution of fundamental niches. *Evolutionary Ecology*, 6:433–447.

Houle G. 2008. Plant species richness and its determinants on a coastal dune system at Îles de la Madeleine, Québec (Canada). *Ecoscience,* 15:113–120.

Howe HF, Smallwood J. 1982. Ecology of seed dispersal. *Annual Review of Ecology and Systematics,* 13:201–228.

Hsiao TC. 1973. Plant responses to water stress. *Annual Review of Plant Physiology*, 24:519–570.

Hughes PW, Simons AM. 2014. The continuum between semelparity and iteroparity: plastic expression of parity in response to season length manipulation in *Lobelia inflata*. *BMC Evolutionary Biology*, 14:90.

Hutchinson GE. 1957. Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, 22:415–427.

Kanda LL, Fuller TK, Sievert PR, Kellogg RL. 2009. Seasonal source-sink dynamics at the edge of a species' range. *Ecology,* 90:1574–1585.

Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. *Ecology Letters,* 7:1225–1241.

Kearney TH. 1904. Are plants of sea beaches and dunes true halophytes? *Botanical Gazette*, 37:424–436.

Keddy PA. 1980. Population ecology in an environmental mosaic: *Cakile edentula* on a gravel bar. *Canadian Journal of Botany*, 58:1095–1100.

Keddy PA. 1981. Experimental demography of the sand-dune annual, *Cakile edentula*, growing along an environmental gradient in Nova Scotia. *Journal of Ecology,* 69:615–630.

Keddy PA. 1982. Population ecology on an environmental gradient: *Cakile edentula* on a sand dune. *Oecologia,* 52:348–355.

Khan MA, Ungar IA, Showalter AM. 1999. Effects of salinity on growth, ion content, and osmotic relations in *Halopyrum mucronatum* (L.) Stapf. *Journal of Plant Nutrition*, 22:191–204.

Kirkpatrick M, Barton NH. 1997. Evolution of a species' range. *American Naturalist,* 150:1–23.

Levin DA. 1988. Local differentiation and the breeding structure of plant populations. 305–329 *in* Plant Evolutionary Biology. Chapman & Hall, New York.

levinsh G. 2006. Biological basis of biological diversity: physiological adaptations of plants to heterogeneous habitats along a sea coast. *Acta Universitatis Latviensis*, 710(Biology):53–79.

MacArthur RH. 1972. Geographical Ecology: Patterns in the Distribution of Species. Princeton University Press, New Jersey.

MacMillan MR, and Quijón PA. 2012. Wrack patches and their influence on upper-shore macrofaunal abundance in an Atlantic Canada sandy beach system. *Journal of Sea Research,* 72:28–37.

Marsico TD, Hellmann JJ. 2009. Dispersal limitation inferred from an experimental translocation of *Lomatium* (Apiaceae) species outside their geographic ranges. *Oikos,* 118:1783–1792.

Martin WE. 1959. The vegetation of Island Beach State Park. *Ecological Monographs*, 21:1–46.

Martínez ML, Maun MA. 1999. Responses of dune mosses to experimental burial by sand under natural and greenhouse conditions. *Plant Ecology,* 145:209–219.

Maun MA. 2004. Burial of plants as a selective force in sand dunes. 119–135 *in* ML Martínez and NP Psuty Coastal Dunes, Ecology and Conservation, Springer-Verlag Berlin Heidelberg, New York.

Maun MA. 2009. The Biology of Coastal Sand Dunes. Oxford University Press, Oxford.

Maun MA, Perumal J. 1999. Zonation of vegetation on lacustrine coastal dunes: effects of burial by sand. *Ecology Letters,* 2:14–18.

McHugh P, Budy P. 2005. An experimental evaluation of competitive and thermal effects on brown trout (*Salmo trutta*) and Bonneville cutthroat trout (*Oncorhynchus clarkii utah*) performance along an altitudinal gradient. *Canadian Journal of Fisheries and Aquatic Science,* 62:2784–2795.

McLachlan A, and Brown A. 2006. The Ecology of Sandy Shores: Second Edition. Academic Press, USA.

Megdiche W, Hessini K, Gharbi F, Jaleel CA, Ksouri R. 2008. Photosynthesis and photosystem 2 efficiency of two salt-adapted halophytic seashore *Cakile maritima* ecotypes. *Photosynthetica,* 46:410–419.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, *et al*. 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science,* 15:684–692.

Olsson-Seffer P. 1909. Relation of soil and vegetation on sandy sea shores. *Botanical Gazette*, 47:85–126.

Oosting HJ, Billings WD. 1942. Factors effecting vegetational zonation on coastal dunes. *Ecology,* 23:131–142.

Parida AK, Das AB. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety,* 60:324–349.

Parmesan C, Ryrholm N, Stefanescu C, Hill JK, Thomas CD, Descimon H, Huntley B, Kaila L, Kullberg J, Tammaru T, Tennent WJ, Thomas JA, Warren M. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature,* 399:579–583.

Payne AM, Maun MA. 1984. Reproduction and survivorship of *Cakile edentula* var. *lacustris* along the Lake Huron shoreline. *American Midland Naturalist,* 111:86–95.

Pemadasa MA, Lovell PH. 1974a. Factors affecting the distribution of some annuals in the dune system at Aberffraw, Anglesey. *Journal of Ecology,* 62:403–416.

Pemadasa MA, Lovell PH. 1974b. Interference in populations of some dune annuals. *Journal of Ecology,* 62:855–868.

Pulliam HR. 2000. On the relationship between niche and distribution. *Ecology Letters,* 3:349–361.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Richards CL, White SN, McGuire MA, Franks SJ. 2010. Plasticity, not adaptation to salt level, explains variation along a salinity gradient in a salt marsh perennial. *Estuaries and Coasts*, 33:840–852.

Rodman JE. 1974. Systematics and evolution of the genus *Cakile* (Cruciferae). *Contribution of the Grey Herbarium, Harvard University*, 205:3–146.

Rozema J, Bijl F, Dueck T, Wesselman H. 1982. Salt-spray stimulated growth in strand-line species. *Physiologia Plantarum*, 56:204–210.

Sagarin RD, Gaines SD. 2002. Geographical abundance distributions of coastal invertebrates: using one-dimensional ranges to test biogeographic hypotheses. *Journal of Biogeography,* 29:985–997.

Sambatti JBM, Rice KJ. 2006. Local adaptation, patterns of selection, and gene flow in the Californian serpentine sunflower (*Helianthus exilis*). *Evolution*, 60:696–710.

Samis KE, Eckert CG. 2007. Testing the abundant center model using range-wide demographic surveys of two coastal dune plants. *Ecology,* 88:1747–1758.

Samis KE, Eckert CG. 2009. Ecological correlates of fitness across the northern geographic range limit of a Pacific Coast dune plant. *Ecology,* 90:3051–3061.

Schlichting CD. 1986. The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics,* 17:667–693.

Schmitt J, Dudley SA, Pigliucci M. 1999. Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade-avoidance responses in plants. *American Naturalist*, 153:S43–S54.

Seneca ED, Woodhouse WW, Broome SW. 1976. Dune stabilization with *Panicum amarum* along the North Carolina Coast. *Miscellaneous Report – Coastal Engineering Research Centre (USA),* 77-1:1–42.

Sexton JP, McIntyre PJ, Angert AL, Rice KJ. 2009. Evolution and ecology of species range limits. *Annual Review of Ecology and Systematics,* 40:415–436.

Shafi M, Bakht J, Razuddin, Hayat Y, Zhang GP. 2011. Genotypic difference in the inhibition of photosynthesis and chlorophyll fluorescence by salinity and cadmium stress in wheat. *Journal of Plant Nutrition,* 34:315–323.

Sopinka NM, Hinch SG, Middleton CT, Hills JA, Patterson DA. 2014. Mother knows best, even when stressed? Effects of maternal exposure to a stressor on offspring performance at different life stages in a wild semelparous fish. *Oecologia,* 175:493–500.

St. Lawrence Global Observatory-SLGO. 2003. URL: http://slgo.ca/en/slmonitored/context/variables.html.

Stanton-Geddes J, Tiffin P, Shaw RG. 2012. Role of climate and competitors in limiting fitness across range edges of an annual plant. *Ecology,* 93:1604–1613.

Stinson KA. 2009. Effects of snowmelt timing and neighbor density on the altitudinal distribution of *Potentilla diversifolia* in Western Colorado, U.S.A. *Arctic, Antarctic, and Alpine Research,* 37:379–386.

Storz JF, Scott GR, Cheviron ZA. 2010. Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. *Journal of Experimental Biology,* 213:4125–4136.

Sultan SE. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science,* 5:537–542.

Sultan SE. 2003. Phenotypic plasticity in plants: a case study in ecological development. *Evolution and Development*, 5:25–33.

Sykes MT, Wilson JB. 1988. An experimental investigation into the response of some New Zealand sand dune species to salt spray. *Annals of Botany*, 62:159–166.

Takahashi K, Kamitani T. 2004. Effect of dispersal capacity on forest plant migration at a landscape scale. *Journal of Ecology,* 92:778–785.

Tang CCL. 2007. High-resolution monthly temperature and salinity climatologies for the northwestern North Atlantic Ocean. *Canadian Data Report of Hydrography and Ocean Science,* No. 169:iv–55.

Thiede DA, Augspurger CK. 1996. Intraspecific variation in seed dispersion of *Lepidium campestre* (Brassicaceae). *American Journal of Botany*, 83:856–866.

Valladares F, Gianoli E, Gómez JM. 2007. Ecological limits to plant phenotypic plasticity. *New Phytologist*, 176:749–763.

van Kleunen M, Fischer M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist,* 166:49–60.

Venable DL, Brown JS. 1988. The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. *American Naturalist,* 131:360–384.

Weber MG, Clement WL, Donoghue MJ, Agrawal AA. 2012. Phylogenetic and experimental tests of interactions among mutualistic plant defense traits in *Viburnum* (Adoxaceae). *American Naturalist*, 180:450–463.

Weinig C. 2000. Plasticity versus canalization: Population differences in the timing of shade-avoidance responses. *Evolution*, 54:441–451.

Wender NJ, Polisetty CR, Donohue K. 2005. Density-dependent processes influencing the evolutionary dynamics of dispersal: a functional analysis of seed dispersal in *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany*, 92:960–971.

West BT, Welch KB, Galecki AT. 2007. Linear Mixed Models: A Practical Guide Using Statistical Software. CRC Press, Taylor & Francis Group, USA.

Wetson AM, Zörb C, John EA, Flowers TJ. 2012. High phenotypic plasticity of *Suaeda maritima* observed under hypoxic conditions in relation to its physiological basis. *Annals of Botany*, 109:1027–1036.

Wickham H. 2009. ggplot2: Elegant Graphics for Data Analysis. Springer New York.

Wickham H. 2011. The split-apply-combine strategy for data analysis. *Journal of Statistical Software*, 40:1–29.

Wiens JJ, Graham, CH. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics*, 36:519–539.

Williams DG, Mack RN, Black RA. 1995. Ecophysiology of introduced *Pennisetum setaceum* on Hawaii: The role of phenotypic plasticity. *Ecology,* 76:1569–1580.

Wilson JB, Sykes MT. 1999. Is zonation on coastal sand dunes determined primarily by sand burial or by salt spray? A test in New Zealand dunes. *Ecology Letters,* 2:233–236.

Xu M, Yu S. 2014. Elevational variation in density dependence in a subtropical forest. *Ecology and Evolution*, 4:2823–2833.

Zhang J. 1996. Interactive effects of soil nutrients, moisture and sand burial on the development, physiology, biomass and fitness of *Cakile edentula*. *Annals of Botany*, 78:591–598.

Zhang J, Maun M.A. 1992. Effects of burial in sand on the growth and reproduction of *Cakile edentula*. *Ecography*, 15:296–302.

Zuur A.F, Ieno E.N, Walker N.J, Saveliev A.A, Smith G.M. 2009. Mixed Effects Models and Extensions in Ecology With R. Springer, USA.

Table 2.1: Distal and proximal 2012 fruit collections from beach and dune used for transplant (Section 2.2.3) and greenhouse (Section 2.2.4) experiments with *Cakile edentula*. The number of seed families for the transplant experiment at Darnley, PE and Martinique, NS, with the number of seeds collected/family in brackets, and the number of seeds from each source zone within site used in the greenhouse experiment are shown.

	Transplant exp. families (seeds/family)				Greenhouse exp. total seeds	
	Darnley		Martinique			
Collection Site	Beach	Dune	Beach	Dune	Beach	Dune
Darnley, PE	15 (20)	10 (23)	20 (12-13)	6 (~10)	56	56
Basin Head, PE	10 (20)	10 (20)	–	–	–	–
Martinique, NS	10 (11)	5 (32)	17 (18-19)	5 (~12)	56	56
Total seed	610	590	600	120	112	112

Table 2.2: Summary of linear models (following Zuur *et al*., 2009) used to assess plant traits measured in the three study components: population surveys (Section 2.2.2), transplant experiment (Section 2.2.3), and the greenhouse experiment (Section 2.2.4). The models used on survey/experiment datasets and the transformations applied to response variables within models are described.

	Model	Dataset	Response variables	Fixed effect(s)	Random effect(s)
Population Surveys (random and tagged plants)[1]	Mixed	All population surveys	Log_{10} Fitness, Log_{10} Size, Height, √Branches, Log_{10} Distance to neighbor, Ranked Seed set ratio	Zone	Intercept: Site Slope: Zone x Site
		Tagged plants only	Log_{10} Growth rate, √Flowering duration		
Transplant Experiment (beach and dune at Martinique and Darnley) [2]	Fixed	Martinique transplant site	Log_{10} Lifetime fitness, Growth rate, √Stem length, Ranked Flowering duration	Transplant zone, source site, source zone(source site), and transplant zone x source zone(source site)	NA
		Dune transplant zone	√Lifetime fitness, Growth rate, Ranked Stem length, Ranked Flowering duration	Transplant site, source zone(source site), source site, and transplant site x source zone(source site)	
Greenhouse Experiment[3]	Mixed	–	√Lifetime fitness, √Total branches, Growth rate, Seed set ratio, Stem length, Total stem nodes, Flowering duration[‡], Ranked New leaves, Ranked Change in stem diameter, Water content, Mean change in chlorophyll content	Source zone, density, spray treatment, and their two-way and three-way interactions	Intercept: Site, and Tote

[1] Final random intercept and slope model (Zuur *et al*., 2009) = lmer(response ~ zone + (1 + Zone|Site))

[2] Final model = lm(response ~ TPzone + TPsite + Ssite/Szone + Ssite/Szone:TPzone, data=MNS); lm(response ~ TPsite + Ssite + Ssite/Szone + Ssite/Szone:TPsite, data=Dune)

[3] Final model = lmer(response ~ zone*density*spray+(1|Tote)+(1|Site))

[‡] Replants (as described in Section 2.2.4) were removed from the greenhouse experiment analysis, reducing the sample size.

Table 2.3: Results of linear mixed models assessing heterogeneity in wild *Cakile edentula* traits between zones, while considering the variation in the zone effect among sites (random site intercept and slope model; as per Zuur et al., 2009), in population surveys of random and tagged plants. Likelihood ratio statistic and *P*-value (in parentheses) associated with removing the fixed and random effects from the model (as described in methods) are reported. Significant effects are bolded, and marginally non-significant effects are bolded with grey text.

Response variable	Fixed effects likelihood ratio statistic (*P*)	Random effects likelihood ratio statistic (*P*)	
	Zone (df = 1)	Site[1] (df = 3)	Zone x Site[2] (df = 5)
Log_{10} Fitness	**9.42 (0.0021)**	**157.31 (<0.0001)**	**11.30 (0.0035)**
Log_{10} Size (HxWxL; cm^3)	**9.39 (0.0022)**	**284.46 (<0.0001)**	**25.75 (<0.0001)**
Height (cm)	**5.67 (0.017)**	**152.02 (<0.0001)**	**14.75 (0.00063)**
√No. of Branches	**8.27 (0.0040)**	**163.55 (<0.0001)**	**15.36 (0.00046)**
Log_{10} Dist. to Neighbour (cm)	**10.50 (0.0012)**	**71.92 (<0.0001)**	**75.65 (<0.0001)**
Ranked Seed Set Ratio	0.84 (0.36)	**54.01 (<0.0001)**	**7.15 (0.028)**
Log_{10} Growth Rate (cm^3/day)	1.67 (0.20)	**6.43 (0.046)**	**19.58 (0.00010)**
√Flowering Duration (days)	0.31 (0.58)	**5.32 (0.075)**	1.35 (0.51)

[1] Likelihood ratio test (one-tailed) for variation among sites in the response variable.
[2] Likelihood ratio test (one-tailed) for variation between zones among sites in the response variable.

Table 2.4: Descriptive statistics for traits measured on wild *Cakile edentula* plants in empirical population surveys (tagged and random) by zone (Z) within site (S). Sample sizes (n), mean ± standard error (se), and coefficient of variation (CV) are reported for plant traits measured at senescence within each zone (1 = beach, 2 = dune) by site (AR = Anglo Rustico, PE, BH = Basin Head, PE, DB = Darnley, PE, KB = Kouchibouguac, NB, LH = Little Harbour, PE, MNS = Martinique, NS, TH = Taylor Head, NS). Back-transformed means and standard errors of $\log_{10}$ and square root transformed variables are provided, while the raw mean ± se is given for rank transformed variables. All CVs were calculated using raw data.

		$\log_{10}$ Fitness			$\log_{10}$ Size (cm^3)			Height (cm)			√No. of Branches		
S	Z	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n
AR	1	183.68 + 89.02, - 59.96	111	25	4560 + 13920, - 10664	138	25	29.52 ± 2.037	35	25	10.02 + 0.64, - 0.62	33	25
	2	88.51 + 21.84, - 17.51	94	18	12724 + 4671, - 3416	99	18	23.00 ± 1.83	34	18	6.66 + 0.54, - 0.52	39	18
BH	1	211.56 + 82.3, - 59.25	143	25	26860 + 12827, - 8681	200	25	22.44 ± 1.45	32	25	8.13 + 0.54, - 0.53	36	25
	2	11.98 + 2.66, - 2.18	106	25	328 + 154, - 105	381	25	9.44 ± 1.20	63	25	3.16 + 0.38, - 0.36	72	25
DB	1	12.30 + 3.4, - 2.66	150	18	147 + 60, - 42	176	18	7.50 ± 0.53	30	18	2.81 + 0.36, - 0.34	63	18
	2	3.49 + 0.59, - 0.51	167	33	66 + 21, - 16	208	33	9.18 ± 0.62	39	33	2.17 + 0.22, - 0.21	82	33
KB	1	141.87 + 69.18, - 46.51	101	45	71419 + 16798, - 13533	97	45	26.82 ± 1.69	42	45	6.87 + 0.75, - 0.71	66	45
	2	110.48 + 26.52, - 21.39	117	25	6608 + 1673, - 1335	111	25	15.96 ± 0.99	31	25	6.77 + 0.39, - 0.38	33	25

(Continued…)

Table 2.4: (continued; 2 of 4) Descriptive statistics for wild plant traits measured in the population surveys (tagged and random) by zone (Z) within site (S).

		Log_{10} Fitness			Log_{10} Size (cm^3)			Height (cm)			√No. of Branches		
S	Z	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n
LH	1	220.05 + 85.19, - 61.42	152	19	12486 + 6173, - 4131	250	19	17.32 ± 1.57	40	19	8.28 + 0.65, - 0.62	36	19
	2	77.72 + 18.79, - 15.13	110	30	6689 + 1772, - 1401	119	30	16.03 ± 1.21	41	30	5.95 + 0.43, - 0.41	40	30
MNS	1	171.61 + 23.85, - 20.94	60	10	10311 + 2403, - 1949	74	10	15.90 ± 1.22	24	10	7.45 + 0.39, - 0.38	18	10
	2	12.12 + 2.72, - 2.22	199	20	416 + 157, - 114	304	20	11.90 ± 0.92	34	20	2.41 + 0.40, - 0.37	108	20
TH	1	857.08 + 137.72, - 118.65	81	45	131249 + 24661, - 20760	91	45	29.69 ± 1.24	28	45	11.70 + 0.54, - 0.53	31	45
	2	164.22 + 49.37, - 37.96	141	45	17301 + 7563, - 5262	171	45	25.96 ± 1.63	42	45	8.19 + 0.67, - 0.64	51	45

(Continued…)

Table 2.4: (continued; 3 of 4) Descriptive statistics for wild plant traits measured in the population surveys (tagged and random) by zone (Z) within site (S).

		Log_{10} Dist. to Neighbour (cm)			Ranked Seed Set Ratio			Log_{10} Growth Rate (cm^3/day)			√Flowering Duration (days)		
S	Z	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n
AR	1	120.92 + 12.03, - 10.94	31	25	90.82 ± 6.79	23	20	–	–	–	–	–	–
	2	11.55 + 2.49, - 2.049	108	18	77.13 ± 8.98	32	15	–	–	–	–	–	–
BH	1	90.70 + 17.87, - 14.93	49	25	–	–	–	–	–	–	–	–	–
	2	11.93 + 2.29, - 1.92	96	25	–	–	–	–	–	–	–	–	–
DB	1	8.35 + 1.87, - 1.53	230	18	–	–	–	–	–	–	–	–	–
	2	9.40 + 0.68, - 0.63	52	33	–	–	–	–	–	–	–	–	–
KB	1	75.71 + 12.74, - 10.9	59	25	107.62 ± 3.48	8	20	–	–	–	–	–	–
	2	27.89 + 2.66, - 2.43	41	25	107.07 ± 4.37	11	20	–	–	–	–	–	–

(Continued…)

Table 2.4: (continued; 4 of 4) Descriptive statistics for wild plant traits measured in the population surveys (tagged and random) by zone (Z) within site (S).

		Log_{10} Dist. to Neighbour (cm)			Ranked Seed Set Ratio			Log_{10} Growth Rate (cm^3/day)			√Flowering Duration (days)		
S	Z	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n	Mean ± se	CV (%)	n
LH	1	42.79 + 13.48, - 10.25	88	19	101.22 ± 12.16	59	18	98.33 + 44.38, - 30.58	117	17	40.68 + 5.68, - 5.31	42	18
	2	11.99 + 1.16, - 1.056	54	30	57.70 ± 10.53	83	22	77.85 + 20.85, - 16.45	120	22	31.81 + 5.96, - 5.45	60	22
MNS	1	55.96 + 15.24, - 11.98	77	10	–	–	–	139.72 + 35.25, - 28.15	73	9	16.27 + 4.42, - 3.89	62	9
	2	10.00 + 1, - 0.91	44	20	–	–	–	7.05 + 3.73, - 2.44	268	13	21.64 + 3.98, - 3.65	48	13
TH	1	122.11 + 13.12, - 11.85	31	25	30.07 ± 4.15	61	20	–	–	–	–	–	–
	2	12.48 + 1.44, - 1.29	60	25	45.58 ± 5.70	39	18	–	–	–	–	–	–

Table 2.5: Descriptive statistics for traits measured on *Cakile edentula* plants in the transplant experiment. Mean ± standard error (se), coefficient of variation (CV), and sample sizes (n) are reported for plant traits by (A) transplant site for plants transplanted into the dune, and by (B) transplant zone (beach, dune) for transplants at Martinique. Back-transformed means ± se of log_{10} and square root transformed variables are provided, while the raw mean ± se is given for rank transformed variables. All CVs were calculated using raw data.

		(A) Transplant Site		(B) Transplant Zone	
Trait		Darnley	Martinique	Beach	Dune
Lifetime Fitness [1]	Mean ± se	1.70	3.68	13.04	3.39
		+ 0.52, - 0.45	+ 7.40, - 3.42	+ 14.62, - 6.89	+ 4.87, - 2.00
	CV	46	205	86	205
	n	17	12	15	12
Growth Rate	Mean ± se	0.024 ± 0.0055	0.057 ± 0.011	0.090 ± 0.011	0.057 ± 0.011
	CV	128	102	61	102
	n	30	30	27	30
√Final Stem Length [2]	Mean ± se	4.44 ± 0.26	6.32 ± 1.50	9.79	6.25
				+ 3.42, - 2.91	+ 4.60, - 3.34
	CV	24	79	32	79
	n	17	11	15	11
Ranked Flowering Duration	Mean ± se	6.18 ± 1.77	10.50 ± 4.087	16.80 ± 4.57	10.50 ± 4.087
	CV	118	135	105	135
	n	17	12	15	12

[1] Lifetime fitness variable was Log_{10} transformed for analysis of Martinique transplants, but square root transformed for dune transplant analysis that included both sites.

[2] Final stem length variable was square root transformed for analysis of Martinique transplants, but rank transformed for dune transplant analysis that included both sites.

Table 2.6: Results of linear, fixed effect models assessing heterogeneity in *Cakile edentula* traits between transplant location and seed sources in a transplant experiment conducted at two sites. (A) Transplant sites (TS), source zones within source sites (SZ within SS), source sites (S), and the interaction between transplant site and source zone within source site (TS x SS/SZ) effects were assessed for plants transplanted into the dune zone at both transplant sites, and (B) Transplant zones (TZ), source zones within source sites (SZ within SS), source sites (S), and the interaction between transplant zone and source zone within source site (TZ x SS/SZ) effects were assessed for plants transplanted at Martinique. F-value and *P*-value (in parentheses) associated with removing each effect from the model (as described in methods) are reported. Significant effects are bolded, and marginally non-significant effects are bold in grey. Sample sizes by transplant zone and sites are provided in Table 2.5.

Response Variable	Fixed Effect F value (*P* value)			
(A) Dune	TS (df = 1)	SS (df = 1)	SZ within SS (df = 2)	TS x SS/SZ (df = 2)
√Lifetime Fitness	2.25 (0.15)	0.47 (0.50)	0.21 (0.89)	0.52 (0.67)
Growth Rate (cm/day)	**8.34 (0.0055)**	0.24 (0.62)	0.30 (0.82)	0.36 (0.78)
Ranked Stem Length (cm)	0.40 (0.53)	0.068 (0.80)	0.57 (0.64)	1.031 (0.40)
Ranked Flowering Duration	0.47 (0.50)	0.0009 (0.98)	0.80 (0.50)	1.79 (0.18)
(B) Martinique	TZ (df = 1)	SS (df = 1)	SZ within SS (df = 2)	TZ x SS/SZ (df = 2)
Log_{10} Lifetime Fitness	**16.92 (0.00046)**	1.38 (0.25)	1.39 (0.27)	0.041 (0.96)
Growth Rate (cm/day)	**4.35 (0.042)**	0.12 (0.73)	0.33 (0.80)	0.37 (0.77)
√Stem Length (cm)	**3.57 (0.073)**	0.026 (0.87)	1.37 (0.28)	0.0059 (0.99)
Ranked Flowering Duration	0.27 (0.61)	0.26 (0.61)	1.57 (0.22)	0.62 (0.56)

Table 2.7: Pearson pairwise correlations among plant traits measured on *Cakile edentula* in a greenhouse experiment across spray and density treatments and source zones. Correlation coefficients are below the diagonal and the corresponding *P*-values are above the diagonal. Significant correlations ($P < 0.05$) are bolded. Sample sizes for each variable are provided in the left column (full experiment size = 224). Due to missing values, pairwise correlations were performed using the smallest sample size between the two variables (range from 178-224).

n		√Lifetime Fitness	Stem Length	Ranked Total Branches	Ranked New Leaves/Day	Flowering Duration
219	√Lifetime Fitness	–	**0.00048**	**< 0.0001**	0.34	**< 0.0001**
187	Stem Length	**+ 0.25**	–	0.43	**0.039**	0.26
224	Ranked Total Branches	**+ 0.36**	– 0.058	–	**< 0.0001**	**0.018**
224	Ranked New Leaves/Day	– 0.065	**– 0.15**	**+ 0.45**	–	0.31
178	Flowering Duration	**+ 0.47**	+ 0.084	**+ 0.18**	+ 0.077	–

Table 2.8: Mixed, linear model analysis assessing heterogeneity in individual *Cakile edentula* traits between source zones (Z), spray treatments (S), and density (D), while considering the random effect of source site and tote, in a greenhouse experiment. Likelihood ratio (LR) statistic and *P*-value (in brackets) associated with removing fixed and random effects from the model (as described in methods) are reported. Significant effects are bolded, and marginally non-significant effects are bold in grey.

Response variable	Fixed effects (*P*)							Random effects (*P*)	
	Z	D	S	Z x D	Z x S	D x S	Z x D x S	Site	Tote
√Lifetime fitness	**2.93 (0.087)**	**4.85 (0.028)**	1.38 (0.24)	0.59 (0.44)	0.019 (0.89)	**6.96 (0.0083)**	2.029 (0.15)	0 (1.0)	**5.46 (0.0097)**
√Total branches	**3.021 (0.082)**	1.71 (0.19)	**17.72 (< 0.0001)**	0.0048 (0.94)	2.58 (0.11)	**14.56 (< 0.0001)**	0.0071 (0.93)	0 (1.0)	0 (1.0)
Stem growth rate (cm/day)	**13.77 (0.00021)**	**11.21 (0.00081)**	**11.39 (0.00074)**	**2.91 (0.088)**	**5.23 (0.022)**	0.091 (0.76)	2.24 (0.13)	**3.33 (0.034)**	**8.53 (0.0017)**
Seed set ratio (proximal/distal)	1.23 (0.27)	0.35 (0.55)	0.14 (0.71)	**3.99 (0.046)**	0.070 (0.79)	1.86 (0.17)	0.18 (0.67)	0.64 (0.21)	0.59 (0.22)
Stem length (cm)	**11.46 (0.0009)**	**5.26 (0.023)**	**12.30 (0.0006)**	0.36 (0.55)	**3.18 (0.075)**	1.46 (0.23)	0.12 (0.73)	0 (1.0)	0.13 (0.36)
Total stem nodes	**6.87 (0.0088)**	**2.81 (0.094)**	1.12 (0.29)	1.074 (0.30)	0.095 (0.76)	0.44 (0.51)	1.064 (0.30)	0.37 (0.27)	**2.47 (0.058)**
Flowering duration (days) †	**4.73 (0.030)**	0.23 (0.63)	0.047 (0.83)	0.025 (0.87)	0.60 (0.44)	**2.89 (0.089)**	0.024 (0.88)	**4.42 (0.018)**	**3.22 (0.036)**

(Continued…)

Table 2.8: (continued) Mixed, linear model analysis assessing heterogeneity in individual *Cakile edentula* traits between source zones (Z), spray treatments (S), and density (D), while considering the random effect of source site and tote, in a greenhouse experiment.

Response variable	Fixed effects (*P*)							Random effects (*P*)	
	Z	D	S	Z x D	Z x S	D x S	Z x D x S	Site	Tote
Ranked change in stem diameter (cm)	0.32 (0.57)	**3.25 (0.071)**	**11.50 (0.0007)**	1.037 (0.31)	0.12 (0.73)	0.98 (0.32)	0.41 (0.52)	**7.22 (0.0036)**	**2.019 (0.078)**
Water content (%)	1.48 (0.22)	0.018 (0.89)	**19.77 (< 0.0001)**	1.94 (0.16)	0.33 (0.56)	0.0008 (0.98)	0.39 (0.75)	**20.30 (< 0.0001)**	0 (1.0)
Mean change in chlorophyll content	0.097 (0.75)	**3.99 (0.046)**	**18.17 (< 0.0001)**	1.020 (0.31)	2.17 (0.14)	1.31 (0.25)	1.96 (0.15)	0.35 (0.28)	**2.11 (0.073)**

† Replants were excluded from the model (as described in methods Section 2.2.5).

Table 2.9: Descriptive statistics for traits measured on plants in the greenhouse experiment by source zone (Z), density (D), spray (S), and source site. Mean ± standard error (se), coefficient of variation (CV), and sample sizes (n) of measured traits are provided for significant effects ($p < 0.05$) detected from the mixed model analysis of variance reported in Table 2.8 (with values reported across variables when the interaction or the random effect of site was significant, or for the main effect alone when interactions were not significant). Effects of source zones (1 = beach, 2 = dune), plant density (H = high, L = low), spray treatment (F = freshwater, S = seawater), and site (D = Darnley, PE, M = Martinique, NS) were assessed. Back-transformed mean ± se of square root transformed variables and the arithmetic mean ± se for rank transformed variables are provided. All CVs were calculated using raw data.

Trait	Z	D	S	Site	Mean ± se	Tukey's HSD[1]	CV (%)	n
√Lifetime fitness		H	F		5.38 + 0.45, - 0.44	ac	77	70
		L	F		5.33 + 0.36, - 0.35	ac	48	38
		H	S		2.83 + 0.35, - 0.33	b	97	72
		L	S		6.68 + 0.80, - 0.76	c	70	39
√Total branches		H	F		1.09 + 0.21, - 0.19	ab	283	72
		L	F		0.59 + 0.05, - 0.04	a	323	40
		H	S		1.85 + 0.29, - 0.27	b	158	72
		L	S		3.93 + 0.77, - 0.70	c	116	40
Stem growth rate (cm/day)[2]	1		F	D	0.29 ± 0.013	a	24	25
	2		F	D	0.23 ± 0.013	b	30	26
	1		S	D	0.20 ± 0.012	c	30	28
	2		S	D	0.15 ± 0.015	c	53	27
	1		F	M	0.32 ± 0.016	a	28	29
	2		F	M	0.26 ± 0.017	ac	35	28
	1		S	M	0.18 ± 0.014	b	39	28
	2		S	M	0.21 ± 0.021	bc	52	28
		H		D	0.20 ± 0.010		40	70
		H		M	0.22 ± 0.012		45	72
		L		D	0.25 ± 0.013		32	36
		L		M	0.29 ± 0.015		34	41

(Continued…)

Table 2.9: (continued) Descriptive statistics for traits measured on plants in the greenhouse experiment by source zone (Z), density (D), spray (S), and source site.

Trait	Z	D	S	Site	Mean ± se	Tukey's HSD[1]	CV (%)	n
Seed set ratio (proximal/distal)	1	H			0.59 ± 0.059	a	85	72
	2	H			0.43 ± 0.059	a	116	72
	1	L			0.50 ± 0.060	a	76	40
	2	L			0.60 ± 0.079	a	83	40
Stem length (cm)			F		21.69 ± 0.57		27	106
			S		18.97 ± 0.67		32	81
		H			19.90 ± 0.55		29	113
		L			21.45 ± 0.74		30	74
	1				21.77 ± 0.65		29	98
	2				19.13 ± 0.58		29	89
Total stem nodes	1				8.73 ± 0.20		22	92
	2				8.08 ± 0.20		23	85
Flowering duration (days) [3]	1			D	24.43 ± 4.052		76	21
	2			D	18.03 ± 1.54		46	29
	1			M	31.04 ± 2.62		57	45
	2			M	27.11 ± 2.54		57	37
Ranked new leaves (leaves/day)			F	D	0.009 ± 0.00097		76	55
			F	M	0.009 ± 0.00097		198	57
			S	D	0.024 ± 0.0024		80	56
			S	M	0.017 ± 0.0026		131	56
Ranked change in stem diameter (cm)			F	D	0.022 ± 0.031		928	44
			F	M	-0.014 ± 0.024		-1223	50
			S	D	0.086 ± 0.054		267	18
			S	M	-0.081 ± 0.060		-412	31
Water content (%)			F	D	0.17 ± 0.0040		18	49
			F	M	0.22 ± 0.023		73	51
			S	D	0.12 ± 0.0080		50	53
			S	M	0.16 ± 0.0080		37	54
Mean change in chlorophyll content			F		39.71 ± 0.49		12	102
			S		33.69 ± 0.59		18	106
		H			35.88 ± 0.59		19	134
		L			38.024 ± 0.56		13	74

Table 2.9: (continued) Descriptive statistics for traits measured on plants in the greenhouse experiment by source zone (Z), density (D), spray (S), and source site.

[1] Tukey's HSD multiple comparisons shown using letter reports where treatment levels not sharing the same letter are significantly different at alpha = 0.05. Multiple comparisons were completed for two-way interactions that were significant in linear models presented in Table 2.8. Treatment interactions without letter reports were not significant in linear models.
[2] Tukey's multiple comparisons completed by site, which was a significant random effect in the linear model for this trait, as presented in Table 2.8.
[3] Replants were excluded from the linear model (as described in methods section 2.2.5).

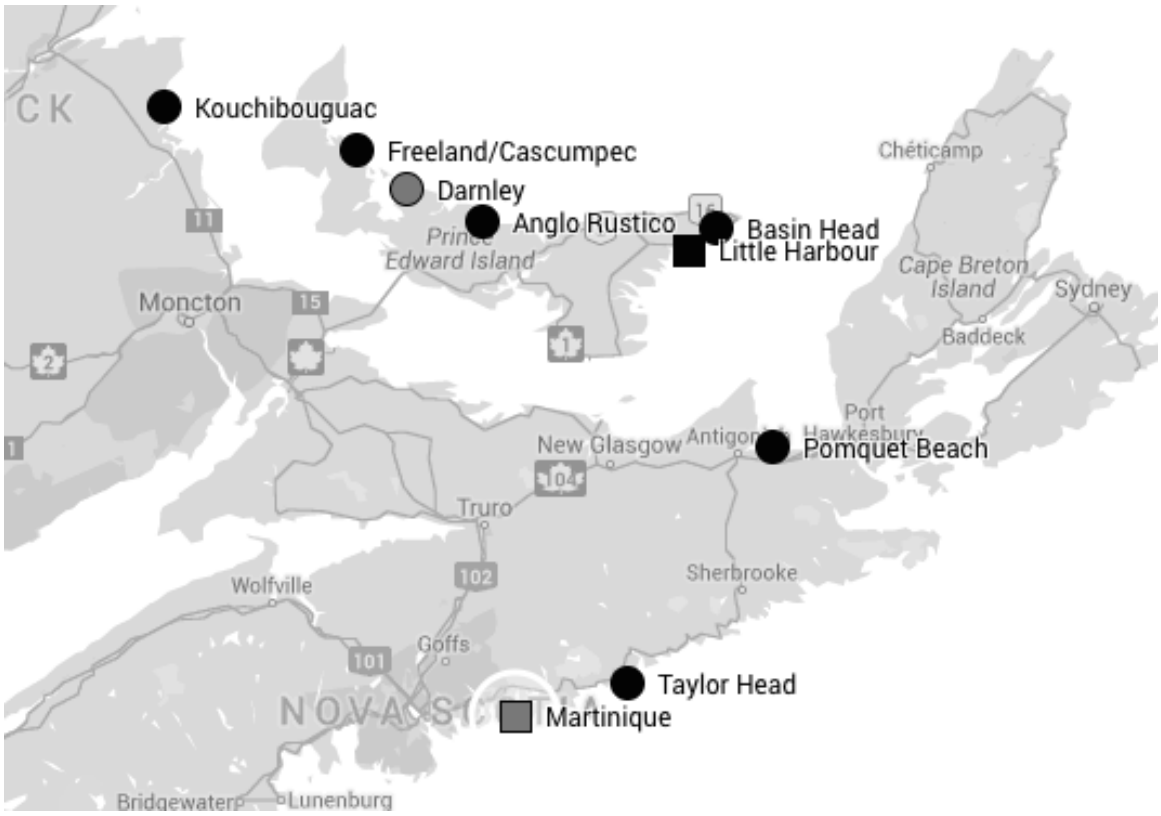


Figure 2.1: Map of coastal *Cakile edentula* populations used for a reciprocal transplant experiment and for empirical surveys of wild plants. Circles represent the seven populations of randomly surveyed plants, squares represent populations of tagged plants that were followed for the entire season, and grey symbols represent the transplant experiment sites. The transplant experiment reciprocally planted seedlings into beach and dune using seeds sourced from Darnley and Basin Head, PE, and Martinique, NS. The greenhouse experiment used seeds sourced from Darnley, PE and Martinique, NS.

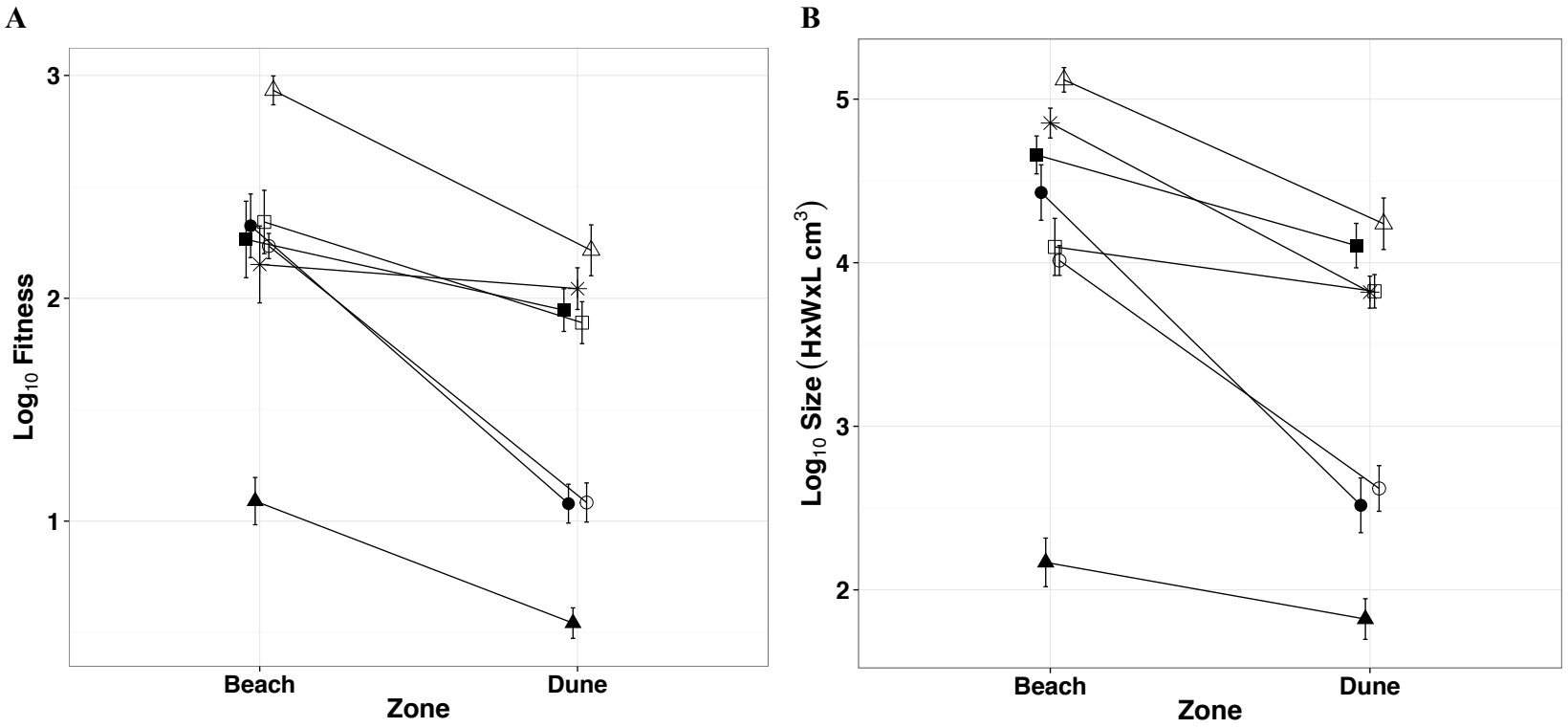


Figure 2.2: The effect of zone on fitness and size of *Cakile edentula* during empirical population surveys in wild populations (tagged and random). All points show mean ± se (filled squares = Anglo Rustico, PE, filled circles = Basin Head, PE, filled triangles = Darnley, PE, asterisk = Kouchibouguac, NB, open squares = Little Harbour, PE, open circles = Martinique, NS, open triangles = Taylor Head, NS), and sample sizes range from 10-45 plants (Table 2.4). A linear, mixed model revealed (A) fitness and (B) size were significantly greater in beach plants than in dune plants. The full model was as described in methods (Section 2.2.5; Table 2.2). See analysis details in Table 2.3.

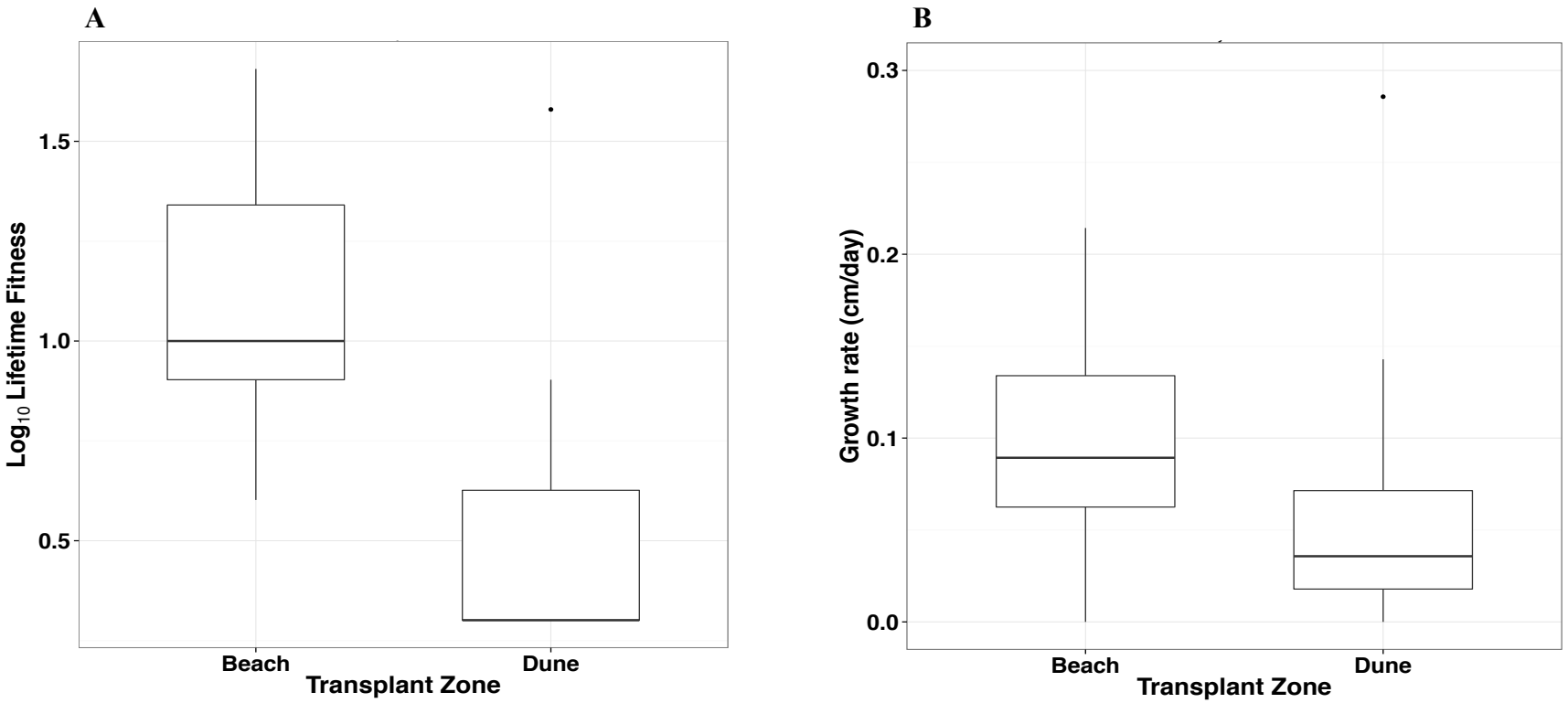


Figure 2.3: The distribution of variation in lifetime fitness (fruit production) and growth rate for experimental transplants of *Cakile edentula* growing at Martinique, NS. Linear, fixed effect models revealed (A) fitness and (B) growth rate were significantly greater in beach plants than in dune plants. The full model was as described in methods section 2.2.5 (Table 2.2). Sample sizes range from 12-30 plants (summarized in Table 2.5). See analysis details in Table 2.6. Within boxes, the middle line equals the median and box edges indicate upper and lower 25% quartiles. The whiskers extend to values within 1.5 x the inter-quartile range, and points beyond the whiskers are outliers.

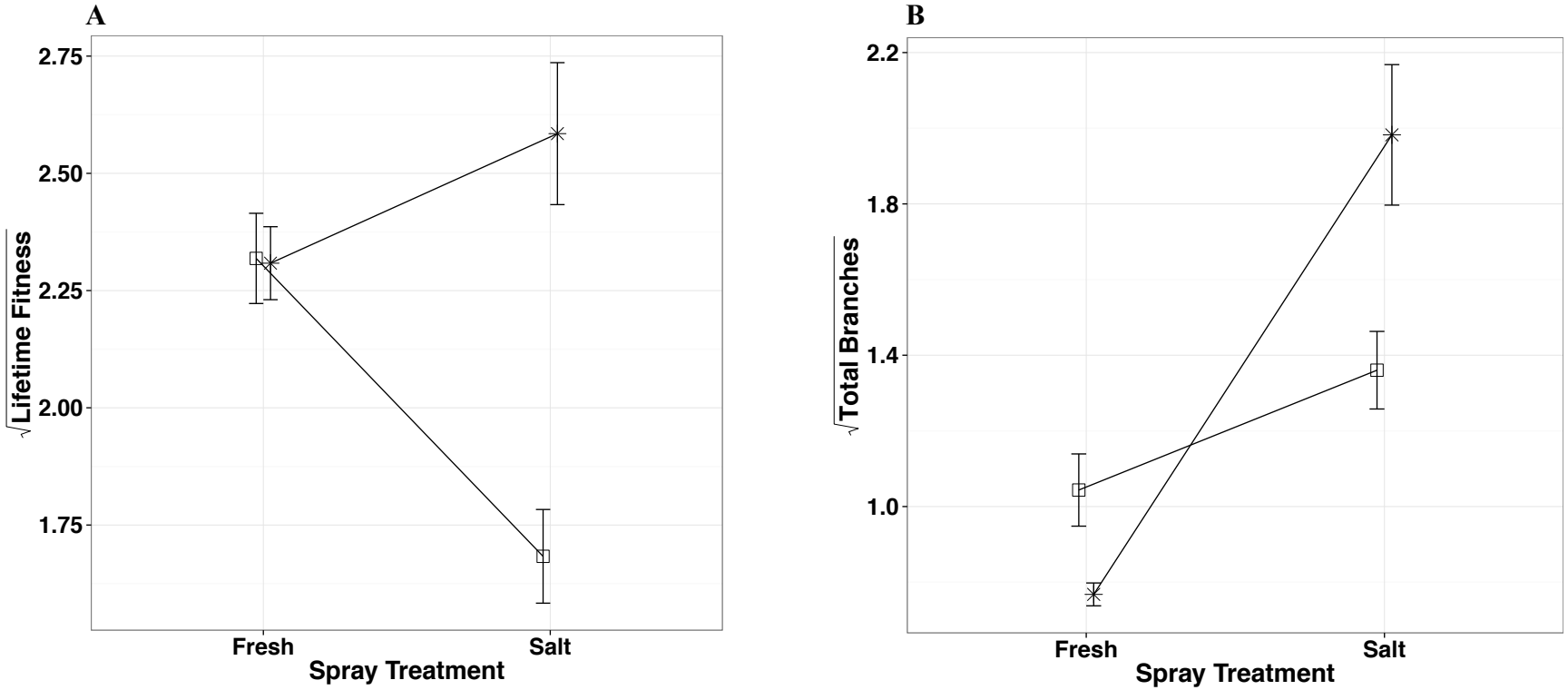


Figure 2.4: The effect of salt spray treatment on lifetime fitness and the number of branches produced by *Cakile edentula* under greenhouse conditions varies significantly between high and low density. All points show mean ± se (square = high density, asterisk = low density), and sample sizes ranged between 38-72 (Table 2.9). Full models are described in the text (Section 2.2.5; Table 2.2). Mixed linear models revealed a significant interaction between spray and density on (A) fitness and (B) total branches. Plant response to treatments did not vary with source zone as a main effect or in any interaction for either trait, and is not shown here. Trait variation among the four spray-by-density treatment levels was assessed using Tukey's HSD tests (Table 2.9): (A) Fitness comparisons between treatments revealed that salt-sprayed plants in high density had lower fitness than the other three treatments. (B) Significantly more branches were produced on salt-sprayed plants in low density than on plants in the other three treatments. See analysis details in Table 2.8.

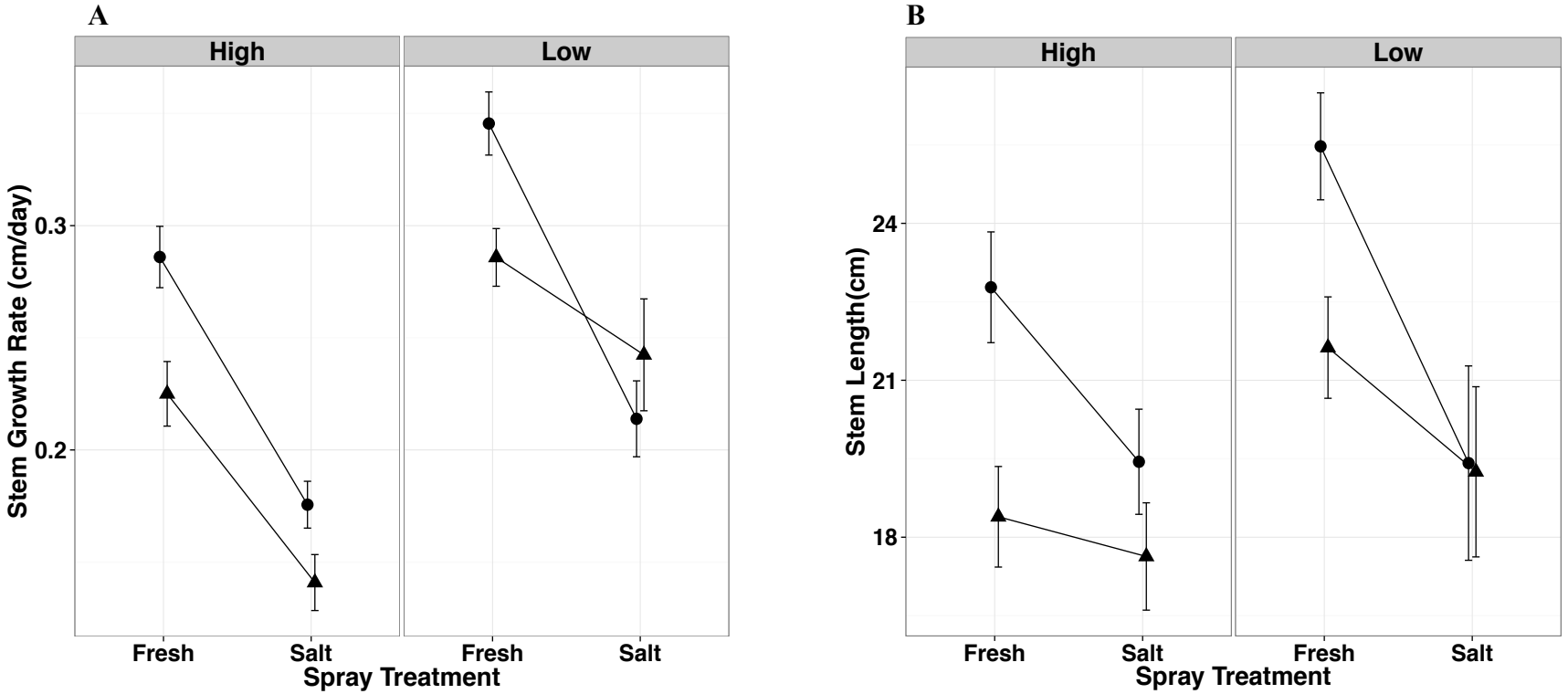


Figure 2.5: The effect of source zone, spray treatment, and density on stem growth rate and stem length of *Cakile edentula* grown in a greenhouse experiment. All points represent mean ± se (circles = beach, triangle = dune), and sample sizes ranged between 18-36 (Table 2.9). Full models are described in the text (Section 2.2.5; Table 2.2). (A) A mixed model revealed a significant interaction between source zone and spray treatment and a significant effect of density on growth rate. Variation among the four zone-by-spray treatment levels was assessed using a Tukey's HSD test (Table 2.9). Dune plants had a significantly lower growth rate then beach plants in the freshwater treatment. Although the effect of site (random) was significant in the model (Table 2.8 and described in the text), the overall patterns were qualitatively similar between sites. (B) Stem length varied significantly by source zone (not shown here), density and spray treatments. Plants treated with seawater or growing in high density had a shorter stem than freshwater-sprayed or low density plants. See additional data from this analysis in Table 2.8.

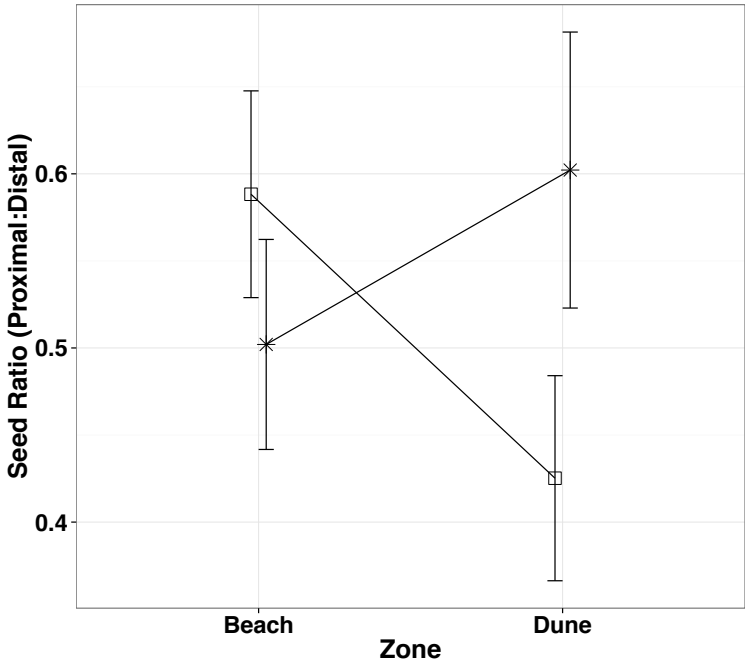
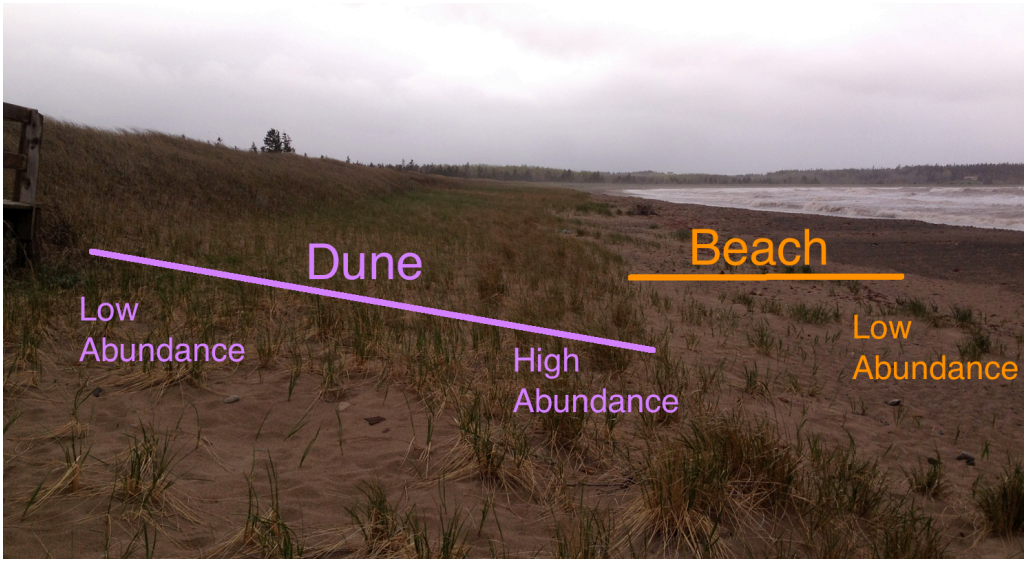


Figure 2.6: The effect of density on proximal to distal seed set ratio of *Cakile edentula* between source zones in a greenhouse experiment. All points represent the mean ± se (square = high density, asterisk = low density), and sample sizes ranged between 40-72 (Table 2.9). A mixed linear model revealed a significant interaction between source zone and density on seed set ratio, however Tukey's multiple comparisons were non-significant (Table 2.9). Full models are described in the text (Section 2.2.5; Table 2.2). Five proximal and five distal fruit were opened for each individual. See additional data from this analysis in Table 2.8.

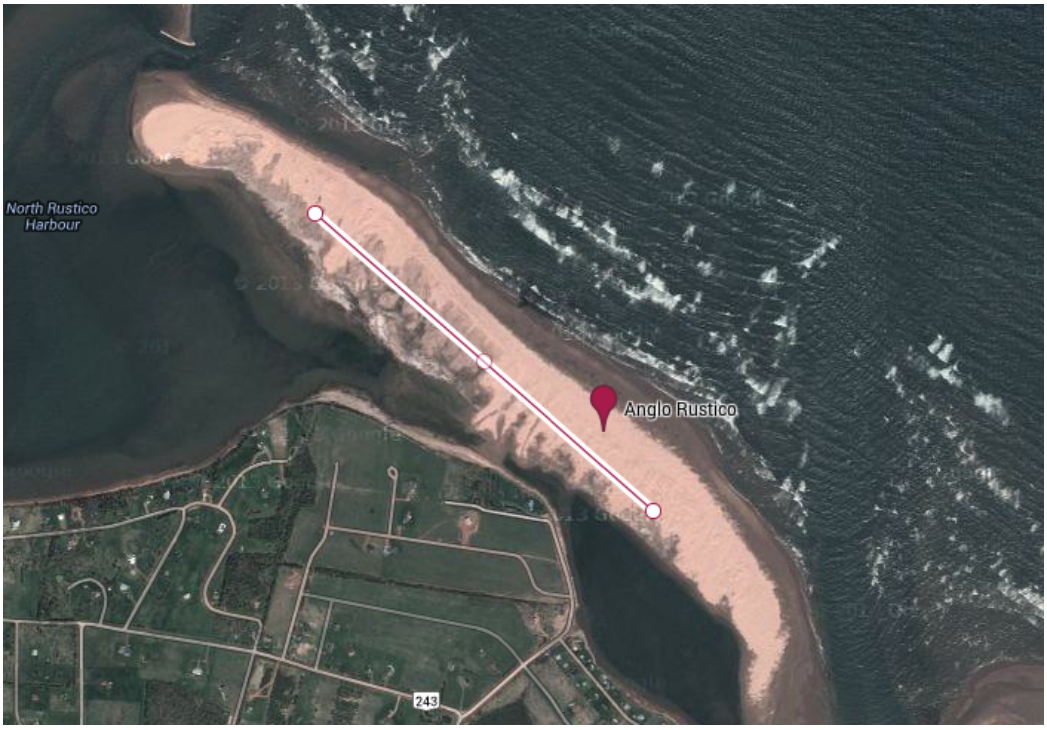
APPENDIX

APPENDIX 2.1: RANDOM AND TAGGED WILD *CAKILE EDENTULA* SURVEY

SITE DESCRIPTIONS



A2.1 Figure 1: General abundance of *Cakile edentula* throughout the species' ecological distribution. Highest abundance of the plant is typically found in the foredune, while abundance in the beach and backdune is less. Photo taken at Pomquet Beach, NS.



A2.1 Figure 2: Anglo Rustico Beach, PE used for random population surveys of *Cakile edentula* in summer 2013. The red line indicates the survey area of approximately 1000 m. Almost completely surrounded by water, Anglo Rustico is a sand spit beach located on the central north coast of Prince Edward Island. The main beach is considered the area exposed to the Gulf of the St. Lawrence, spanning ~750 m in length, and providing a beach to dune gradient running north to south. The Anglo Rustico sand spit separates the mouth of North Rustico Harbour and Rustico Bay. A sand barrier connects the sand spit to mainland and leads to the center of the main beach. The beach is wide (~75 m), with an equally wide dune that covers the remainder of the island. This site is subjected to tidal changes and frequent, ephemeral flooding in lower laying areas of the beach, including the tips. The 1-3 m high dune is fairly uniform from one end to the other, comprised of mostly dune grasses, and contains sparse embryo dunes that form ~10 m in front of the dune ridge. *Cakile edentula* occurs up to 8 m into the dune from the vegetation line on the beach. Overall, the vegetated region on the spit runs 30-75 m from the high tide line. The beach vegetation appears well distributed across the entire habitat, with very few high-density clumps.

A2.1 Figure 3: The portion of Basin Head Beach, PE used for random population surveys of *Cakile edentula* in summer 2013. The red line indicates the survey area of approximately 1000 m. Basin Head is a barrier beach located on the northeast coast of Prince Edward Island, at the mouth of the Northumberland Strait to the Gulf of the St. Lawrence. The beach to dune gradient running east to west, with the public entrance at the south end. For the first 300 m, the 1-4 m high dune ridge is mainly comprised of trees, shrubs and sparse clumps of grasses, with various fungi and mosses inhabiting the back dune. Several embryo dunes emerge in front of this dune ridge. *Cakile edentula* primarily occurs on the face of the dune and in embryo dunes in this region of Basin Head. The remaining 500+ m of Basin Head has a very different appearance. The steep 4-8 m high dune ridge is primarily inhabited by grasses that extend further into the back dune than the previous segment of this site, however *C. edentula* was only observed up to 6 m into this dune type. Overall at Basin Head, the beach starts fairly wide (~35 m) with more dense vegetation (mainly the study system) at the entrance and moves to a narrower strand (~15 m) and more sparse vegetation with the change in dune morphology. Fewer embryo dunes are found in the second morphology segment of this site. Basin Heads beach continues for another 3 km beyond the region included in study (not shown here).

A2.1 Figure 4: Darnley Beach, PE used for random population surveys and a transplant experiment with *Cakile edentula* in summer 2013. The red line indicates the survey area of approximately 500 m. Darnley is a sand-spit on the north coast of Prince Edward Island at the mouth of the Darnley Basin on the east side of Malpeque Bay. The beach to dune gradient runs northwest to southeast, with the beach exposed to the Gulf of the St. Lawrence. The main entrance for the beach passes through an agricultural field at the east end of the beach. Darnley is comprised of three main morphology segments. The first segment at the east end of the beach includes a sharp to gradual 1-3 m dune ridge, likely created by seasonal wave action, with a densely vegetated dune on top. *C. edentula* was not often observed in this segment of dune. Moving west, the second segment includes a more gradual slope or a flat transition between beach and dune. This segment has an area of low-density vegetation (~10-15 m wide) that transitions into high-density (~10-20 m wide) and in front of the backdune. Few embryo dunes occur ~5-10 m in front of this segment. The highest *C. edentula* abundance was found within the first 5 m of this dune, and in patches up to 20 m inland. Finally, the third segment includes many highly vegetated dune mounds and several "blowout" areas that consist of a rockier substrate and few grasses. *Cakile edentula* was observed growing sparsely on the mounds, and fairly dense in the blowout areas.

A2.1 Figure 5: The portion of Freeland/Cascumpec Sandhills Beach, PE used for random population surveys of *Cakile edentula* in summer 2013. The red line indicates the survey area of approximately 500 m. The Cascumpec Sandhills consist of several barrier islands off the north coast of western Prince Edward Island. The area surveyed for this research included a 500 m segment northeast of Frederick Cove in Freeland, between two areas subjected to frequent flooding. The beach to dune gradient runs northeast to southwest, with access by boat and no visible public entrance. This site, referred in the main text as Freeland, included 3-6 m high dune and numerous sandy mounds. The face of the dune ridge was sparsely vegetated with primarily dune grasses, and the mounds varied from high to low-density vegetation. The transition from beach to dune was fairly askew across the surveyed area. The study system was found to occur up to 8 m into the dune. In addition, the beach was consistent in size (~15 m), with the highest abundance of *C. edentula* occurring within 5 m of the dune. Sandhills barrier island extends approximately 6 km.

A2.1 Figure 6: The portion of Kouchibouguac North Beach, NB used for random population surveys of *Cakile edentula* in summer 2013. The red line indicates the survey area of approximately 1000 m. Kouchibouguac North Beach is a barrier island off the northeast coast of New Brunswick, at the mouth of the Northumberland Strait. The beach to dune gradient runs northeast to southwest, with access by boat and no visible public entrance. This site had 3-6 m high dune ridges. The face of the dune ridge was lightly vegetated with primarily dune grasses that extended ~3-5 m onto the beach, with few embryo dunes observed. *Cakile edentula* was found to occur on the dune face and in the extended dune. The beach was consistent in size (~35 m), with the highest abundance of the study system occurring within 10 m of the dune. Kouchibouguac North Beach extends approximately 7 km.

A2.1 Figure 7: Little Harbour Beach, PE used for tagged plant population surveys with *Cakile edentula* in summer 2013. The red line indicates the survey area of approximately 1000 m. Little Harbour is a beach located in front of a bird sanctuary on the northeast coast of Prince Edward Island, between two river mouths. The beach to dune gradient runs southeast to northwest, with public access from the northwest onto the middle of the beach. This site, approximately 1000 m long, consisted of one dune morphology that included 1-5 m high dune ridges. The face of the dune ridge was sparsely vegetated with primarily dune grasses, and several embryo dunes within 10 m of the dune towards the northeastern end of the site. *Cakile edentula* was found occurring 8 m into the dune. The beach varied in width from ~10 m to 35 m, with the highest abundance of *C. edentula* occurring 5-10 m of the dune.

A2.1 Figure 8: The portion of Martinique Beach, NS used for tagged plant population surveys and transplant experiment with *Cakile edentula*, in summer 2013. The red line indicates the survey area of approximately 1000 m. Martinique is a barrier beach (sand spit) in front of a large lagoon comprising part of the Musquodoboit Harbour on the south coast of central Nova Scotia. The beach to dune gradient runs south to north, with the primary public access through several developed (wooden structures) dune cutouts within the first 300 m of the site. The study area consisted of a fairly uniform dune system, with highly vegetated 2-5 m wave-cut dune ridges. The vegetation on these ridges was a mixture of dune grasses, shrubs, and small flowering annuals. *Cakile edentula* often occurred only within the first meter of the dune, which was believed to be due to the high density of other vegetation. The beach was fairly consistent in size, approximately 12 m wide at high tide, with *C. edentula* occurring within 5 m from the edge of the foredune. Martinique Beach sand spit extends approximately 3.5 km.

A2.1 Figure 9: The portion of Pomquet Beach, NS used for random population surveys of *Cakile edentula*, in summer 2013. The red line indicates the survey area of approximately 1000 m. Pomquet is a barrier beach in front of Pomquet Harbour on the north coast of eastern Nova Scotia (between mainland Nova Scotia and Cape Breton). The beach to dune gradient faces north to south, with public access through several wooden boardwalks along the length of the site. The study area consisted of a fairly uniform dune system, with a less than 1 m high dune ridge that gradually transitions from low to high-density vegetation, primarily dune grasses. *Cakile edentula* was observed occurring up to 3 m into the dune. The beach was fairly consistent in size, approximately 25 m wide, with *C. edentula* occurring within 10 m of the dune. Pomquet beach extends approximately 4km.

A2.1 Figure 10: Taylor Head Beach, NS used for random population surveys of *Cakile edentula,* in summer 2013. The red line indicates the survey area of approximately 500 m. Taylor's Head is a protected, pocket beach (not directly exposed to the Atlantic Ocean) along Taylors Head Bay on the south coast of central Nova Scotia. The beach to dune gradient faces east to west, with public access through wooden boardwalks throughout the site, ~1000 m. This site is highly variable from one end to the other, with three distinct morphologies. The first segment starts at the south end of the site, and has a very rocky substrate. The 2 m high dune ridge has a severe transition from sparse dune grasses in front of the ridge (~3 m) to high-density vegetation (dune grasses, low-laying annuals, and shrubs) on top of the ridge, possibly due to the substrate type. *Cakile edentula* was found to occur only at the base of the ridge in the sparse grasses. The second segment, north of the first, had a sandier substrate, with a lesser slope than the first dune morphology. The transition between low-density grasses to dense vegetation was still quite severe, but this dune segment had a wider low-density area (~8 m) than the first. *Cakile edentula* was observed occurring mostly in the low-density area, with very large plants, comparable to the beach, invading sparse grass habitat. Finally, the third segment had a gradual transition from low-density to high-density vegetation in the dune, with the low-density area approximately 5 m wide. *Cakile edentula* was found to grow in the low-density area and about 1 m into the high-density dune.

APPENDIX 2.2 DETAILED DESCRIPTION OF A RECIPROCAL TRANSPLANT EXPERIMENT DESIGNED WITH *CAKILE EDENTULA*.

The following methods have been described to provide a record of the decisions that were made and experimental techniques that were intended to assess the impact of ecological and demographic variables on the survival and fitness of *C. edentula* under standardized conditions in the plant's native habitat. However, due to low survival, the full design was not analyzed (see details of survival in main text, section 2.2.3.1).

Randomly selected fruits (distal and proximal) were collected as per methods outlined in section 2.2.2.1 from wild beach and dune plants in fall 2012 (Table 2.1). All seeds collected from one plant represent one maternal family and are presumed to be full-siblings (maternal plant is the ovule and pollen donor with a primarily selfing mating system; see 2.2.1). Seeds were stored in paper envelopes in the cold and dark, and were scarified (with a scalpel) prior to cold, wet stratification in the dark at 4 ºC for three days in a refrigerator. After manually removing seed coats, germinated seedlings were planted in plastic plug trays (128 2 cm^2 wells) filled with sand collected from the transplant site (Darnley or Martinique) in spring 2013. Trays were placed in a Conviron A1000 germination cabinet for 10 days while emerging, moved to the greenhouse for an additional 12 days, and then hardened at each transplant site for 8-9 days before planting. Seedlings were transplanted in late June into 80 cm x 60 cm experimental transplant blocks with minimal disruption to native vegetation and substrate. Locations for experimental blocks were randomly selected using similar methods described in section 2.2.2.1. At the Darnley transplant site, experimental plants were initially subjected to one of four planned treatments: control, high-density, blocked sea spray and burial, and at

Martinique one of two treatments: control and high-density with ten plots/treatment/zone at each site. Plots in all treatments, excluding high-density treatment (24 transplants at 10 cm spacing), were planted at low-density (12 transplants at 20 cm spacing (selected based on previous research of density-dependent effects; Keddy, 1981). Plants from the same site and zone source were not placed next to each other to avoid the negative effects of sibling competition (Dudley and File, 2007). Experimental plots were spaced at least 5 m apart along a 300 m section running approximately parallel to the shoreline within each zone. In total, 1200 plants were transplanted into 40 experimental plots per beach and dune zone at Darnley, and 720 plants were transplanted into 20 experimental plots per zone at Martinique.

The blocked sea spray treatment consisted of plots where plants were shielded from potential sea spray using a 1 m wide by 0.4 m high screen made of wood supports and garden fabric that were assembled in a "v-shape" 0.3 m in front (to avoid shading plants) of 10 low-density plots in each zone. Squares of cheesecloth (20 cm^2) were posted on wood frames within and in front of randomly selected blocked sea spray treatment plots on the beach and in the dune at Darnley, PE to confirm the screens were blocking sea spray. The cheesecloth assessed for intercepted sodium chloride (most abundant salt in seawater). Six frame pairs were placed in each zone at plant height (~20 cm) for five days. After exposure, a 1 dm^2 section from the middle of the cheese cloth was removed, placed in a clean, sealed bag and stored cold (~4 ºC) and dark until analysis. The cloth section was soaked in 200 mL of distilled water for 10 minutes, with slight agitation, to extract any salt collected on the cloth. Sample conductivity was measured using a VWR®

Symphony Bench Top Meter with Traceable Conductivity Standard (VWR, Pennsylvania, USA). Due to collection errors, these data were not analyzed.

The sand burial treatment consisted of compostable pots (8 cm high, open from top and bottom) placed over all plants within each sand burial treatment plot/zone that were filled with sand, creating a 6 cm burial depth. Plants were buried for three weeks to simulate natural sand burial (based on B.T. Cole *personal observations;* Zhang and Maun, 1992). After burial, the measurements taken for plants in this treatment were different than for other treatments. The date a plant emerged from burial was recorded along with the measured height above the burial sand surface. Plants were uncovered (pots removed and sand lightly dusted away) after 21 days of burial and survival was recorded. All measurements after the date uncovered followed the same outline as other treatments in this experiment. Due to low survival, analysis was not performed on these data.

APPENDIX 2.3: PAIRWISE CORRELATIONS AMONG *CAKILE EDENTULA* TRAITS MEASURED IN RANDOM AND TAGGED POPULATION SURVEYS.

Pearson correlation analysis was used to assess the association between measured plant traits from the population surveys (Section 2.2.2). Significant associations found between traits suggested plants that were larger, grew taller, and had more branches produced more fruit than plants that were smaller and less branchy. The results of all population survey correlation analyses are provided below for each site (Anglo Rustico, Basin Head, Darnley, Kouchibouguac, Little Harbour, and Martinique).

A2.3 Table 1: Pearson pairwise correlations among *Cakile edentula* traits measured in random population surveys at Anglo Rustico, PE between zones. Correlation coefficients are reported below the diagonal and the corresponding *P*-values are above the diagonal. Significant correlations ($P \leq 0.05$) are bolded, and marginally non-significant ($0.1 > P > 0.05$) correlations are bolded with grey text. Sample sizes are provided in Table 2.4, and range from 15 to 25.

Beach	Log_{10} Fitness	Log_{10} Size	Ranked Height	Ranked Branches
Log_{10} Fitness	–	**0.0067**	**0.053**	**0.00076**
Log_{10} Size	**+0.53**	–	**0.0001**	**0.0001**
Ranked Height	**+0.39**	**+0.91**	–	**0.00023**
Ranked Branches	**+0.63**	**+0.78**	**+0.67**	–
Dune	Ranked Fitness	Ranked Size	Ranked Height	Ranked Branches
Ranked Fitness	–	**0.0001**	**0.0038**	**0.0020**
Ranked Size	**+0.87**	–	**0.0001**	**0.00081**
Ranked Height	**+0.65**	**+0.83**	–	**0.0053**
Ranked Branches	**+0.68**	**+0.72**	**+0.63**	–

A2.3 Table 2: Pearson pairwise correlations among *Cakile edentula* traits measured in random population surveys at Basin Head, PE between zones. Correlation coefficients are reported below the diagonal and the corresponding *P*-values are above the diagonal. Significant correlations ($P \leq 0.05$) are bolded. Sample size was 25 (Table 2.4).

Beach	Log_{10} Fitness	Ranked Size	Log_{10} Height	Ranked Branches
Log_{10} Fitness	–	**0.0001**	**0.0001**	**0.0001**
Ranked Size	**+0.90**	–	**0.0001**	**0.0001**
Log_{10} Height	**+0.92**	**+0.92**	–	**0.0001**
Ranked Branches	**+0.82**	**+0.77**	**+0.79**	–
Dune	Ranked Fitness	Log_{10} Size	Ranked Height	Ranked Branches
Ranked Fitness	–	**0.0001**	**0.0001**	**0.0001**
Log_{10} Size	**+0.87**	–	**0.0001**	**0.0001**
Ranked Height	**+0.79**	**+0.78**	–	**0.00042**
Ranked Branches	**+0.88**	**+0.86**	**+0.65**	–

A2.3 Table 3: Pearson pairwise correlations among *Cakile edentula* traits measured in random population surveys at Darnley, PE between zones. Correlation coefficients are reported below the diagonal and the corresponding *P*-values are above the diagonal. Significant correlations ($P \leq 0.05$) are bolded, and marginally non-significant ($0.1 > P > 0.05$) correlations are bolded with grey text. Sample sizes are provided in Table 2.4, and range from 18 to 33.

Beach	Ranked Fitness	Ranked Size	Ranked Height	Ranked Branches
Ranked Fitness	–	**0.0001**	**0.092**	**0.0001**
Ranked Size	**+0.81**	–	**0.0033**	**0.00011**
Ranked Height	**+0.41**	**+0.65**	–	**0.047**
Ranked Branches	**+0.94**	**+0.78**	**+0.47**	–
Dune	Ranked Fitness	Ranked Size	Log_{10} Height	Ranked Branches
Ranked Fitness	–	**0.053**	**0.050**	**0.013**
Ranked Size	**+0.34**	–	**0.0001**	**0.0001**
Log_{10} Height	**+0.34**	**+0.63**	–	**0.00068**
Ranked Branches	**+0.43**	**+0.73**	**+0.56**	–

A2.3 Table 4: Pearson pairwise correlations among *Cakile edentula* traits measured in random population surveys at Kouchibouguac, NB between zones. Correlation coefficients are reported below the diagonal and the corresponding *P*-values are above the diagonal. Significant correlations ($P \leq 0.05$) are bolded, and marginally non-significant ($0.1 > P > 0.05$) correlations are bolded with grey text. Sample sizes are provided in Table 2.4, and range from 20 to 45.

Beach	Ranked Fitness	Log_{10} Size	Log_{10} Height	Ranked Branches
Ranked Fitness	–	**0.0001**	**0.0001**	**0.0001**
Log_{10} Size	**+0.86**	–	**0.0001**	**0.0001**
Log_{10} Height	**+0.59**	**+0.79**	–	**0.0030**
Ranked Branches	**+0.82**	**+0.76**	**+0.43**	–

Dune	Log_{10} Fitness	Ranked Size	Ranked Height	Ranked Branches
Log_{10} Fitness	–	**0.0001**	**0.00036**	**0.0023**
Ranked Size	**+0.78**	–	**0.0001**	**0.0007**
Ranked Height	**+0.66**	**+0.91**	–	**0.0035**
Ranked Branches	**+0.58**	**+0.63**	**+0.56**	–

A2.3 Table 5: Pearson pairwise correlations among *Cakile edentula* traits measured in tagged plant population surveys at Little Harbour, PE between zones. Correlation coefficients are reported below the diagonal and the corresponding *P*-values are above the diagonal. Significant correlations ($P \leq 0.05$) are bolded. Sample sizes are provided in Table 2.4, and range from 17 to 30.

Beach	Log_{10} Fitness	Ranked Size	Ranked Height	Log_{10} Branches
Log_{10} Fitness	–	**0.0001**	**0.0001**	**0.0001**
Ranked Size	**+0.95**	–	**0.0001**	**0.0001**
Ranked Height	**+0.94**	**+0.96**	–	**0.0001**
Log_{10} Branches	**+0.88**	**+0.90**	**+0.87**	–

Dune	Ranked Fitness	Ranked Size	Log_{10} Height	Ranked Branches
Ranked Fitness	–	**0.0001**	**0.033**	**0.0001**
Ranked Size	**+0.83**	–	**0.0001**	**0.00016**
Log_{10} Height	**+0.39**	**+0.67**	–	**0.011**
Ranked Branches	**+0.65**	**+0.63**	**+0.46**	–

A2.3 Table 6: Pearson pairwise correlations among *Cakile edentula* traits measured in tagged plant population surveys at Martinique, NS between zones. Correlation coefficients are reported below the diagonal and the corresponding *P*-values are above the diagonal. Significant correlations ($P \leq 0.05$) are bolded, and marginally non-significant ($0.1 > P > 0.05$) correlations are bolded with grey text. Sample sizes are provided in Table 2.4, and range from 9 to 20.

Beach	Ranked Fitness	Ranked Size	Ranked Height	Ranked Branches
Ranked Fitness	–	0.29	0.92	**0.025**
Ranked Size	+0.37	–	**0.072**	**0.0502**
Ranked Height	+0.037	**+0.59**	–	0.602
Ranked Branches	**+0.70**	**+0.63**	+0.19	–
Dune	Log_{10} Fitness	Log_{10} Size	Log_{10} Height	Ranked Branches
Log_{10} Fitness	–	**0.0001**	**0.00023**	**0.00094**
Log_{10} Size	**+0.84**	–	**0.00056**	**0.0013**
Log_{10} Height	**+0.73**	**+0.702**	–	**0.0017**
Ranked Branches	**+0.68**	**+0.67**	**+0.66**	–

A2.3 Table 7: Pearson pairwise correlations among *Cakile edentula* traits measured in random population surveys at Taylor Head, PE between zones. Correlation coefficients are reported below the diagonal and the corresponding *P*-values are above the diagonal. Significant correlations ($P \leq 0.05$) are bolded. Sample sizes are provided in Table 2.4, and range from 18 to 45.

Beach	Log_{10} Fitness	Log_{10} Size	Height	Log_{10} Branches
Log_{10} Fitness	–	**0.0001**	**0.0001**	**0.0001**
Log_{10} Size	**+0.91**	–	**0.0001**	**0.0001**
Height	**+0.77**	**+0.89**	–	**0.0001**
Log_{10} Branches	**+0.73**	**+0.78**	**+0.56**	–
Dune	Ranked Fitness	Ranked Size	Log_{10} Height	Branches
Ranked Fitness	–	**0.0001**	**0.0001**	**0.0001**
Ranked Size	**+0.96**	–	**0.0001**	**0.0001**
Log_{10} Height	**+0.84**	**+0.903**	–	**0.0001**
Branches	**+0.86**	**+0.86**	**+ 0.78**	–

APPENDIX 2.4: MULTIPLE COMPARISONS BETWEEN SIGNIFICANT LINEAR MODEL INTERACTIONS FOR TRAITS MEASURED ON GREENHOUSE EXPERIMENT PLANTS.

A2.4 Table 1: Tukey's Honest Significant Differences comparisons between significant source zone (Z), density (D), and spray (S) two-way interactions (Table 2.8) for traits measured on plants in the greenhouse experiment. The difference in observed means of source zones (1 = beach, 2 = dune), plant density (H = high, L = low), spray treatment (F = freshwater, S = seawater) interactions are provided along with the p-value. For the growth rate trait, comparisons were done by site (D = Darnley, M = Martinique). Significantly different comparisons are bolded.

Trait	Comparison	Difference		P-value	
√Lifetime fitness	L:F – H:F	–0.010		1.0	
	H:S – H:F	–0.63		<0.0001	
	L:S – H:F	0.27		0.35	
	H:S – L:F	–0.62		0.00077	
	L:S – L:F	0.28		0.43	
	L:S – H:S	0.90		<0.0001	
√Total branches	L:F – H:F	–0.28		0.35	
	H:S – H:F	0.32		0.11	
	L:S – H:F	0.94		<0.0001	
	H:S – L:F	0.59		0.0024	
	L:S – L:F	1.21		<0.0001	
	L:S – H:S	0.62		0.0013	
Stem growth rate		D	M	D	M
	2:F – 1:F	–0.057	–0.058	0.021	0.079
	1:S – 1:F	–0.087	–0.14	<0.0001	<0.0001
	2:S – 1:F	–0.14	–0.12	<0.0001	<0.0001
	1:S – 2:F	–0.030	–0.085	0.38	0.0038
	2:S – 2:F	–0.085	–0.058	0.00012	0.084
	2:S – 1:S	–0.054	0.027	0.022	0.69
Seed Set Ratio	2:H – 1:H	–0.16		0.18	
	1:L – 1:H	–0.086		0.80	
	2:L – 1:H	0.014		1.0	
	1:L – 2:H	0.077		0.85	
	2:L – 2:H	0.18		0.25	
	2:L – 1:L	0.10		0.79	